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21 February 2019

Version of attached file:

Accepted Version

Peer-review status of attached file:

Peer-reviewed

Citation for published item:

Uwineza, Alice and Kalligeraki, Alexia A. and Hamada, Nobuyuki and Jarrin, Miguel and Quinlan, Roy A. (2019) 'Cataractogenic load – a concept to study the contribution of ionizing radiation to accelerated aging in the eye lens.', *Mutation research/reviews in mutation research.*, 779 . pp. 68-81.

Further information on publisher's website:

<https://doi.org/10.1016/j.mrrev.2019.02.004>

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Title: Cataractogenic load – a concept to study the contribution of ionizing radiation to accelerated aging in the eye lens

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PII: S1383-5742(18)30081-4
DOI: <https://doi.org/10.1016/j.mrrev.2019.02.004>
Reference: MUTREV 8264

To appear in: *Mutation Research*

Received date: 18 August 2018
Revised date: 12 February 2019
Accepted date: 14 February 2019

Please cite this article as: Uwineza A, Kalligeraki AA, Hamada N, Jarrin M, Quinlan RA, Cataractogenic load – a concept to study the contribution of ionizing radiation to accelerated aging in the eye lens, *Mutation Research-Reviews in Mutation Research* (2019), <https://doi.org/10.1016/j.mrrev.2019.02.004>

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CATARACTOGENIC LOAD – A CONCEPT TO STUDY THE CONTRIBUTION OF IONIZING RADIATION TO ACCELERATED AGING IN THE EYE LENS

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Competing Interests: The authors have no competing interests to declare.

ABSTRACT

Ionizing radiation (IR) damages DNA and other macromolecules, including proteins and lipids. Most cell types can repair DNA damage and cycle continuously their macromolecules as a mechanism to remove defective proteins and lipids. In those cells that lack nuclei and other organelles, such as lens fiber cells and mammalian erythrocytes, IR-induced damage to macromolecules is retained because they cannot be easily replenished. Whilst the life span for an erythrocyte is several months, the life span of a human lens is decades. There is very limited turnover in lens macromolecules, therefore the aging process greatly impacts lens structure and function over its lifetime. The lens is a tissue where biomolecular longevity, lifelong retention of its components and continued growth are integral to its homeostasis. These characteristics make the lens an excellent model to study the contribution of retained macromolecular damage over time. Epidemiological data have revealed a significant association between exposure to IR, the loss of lens optical function and the formation of cataracts (cataractogenesis) later in life. Lifestyle, genetic and environmental factors all contribute to cataractogenesis due to their effect on the aging process. Cataract is an iconic age-related disease in humans. IR is a recognised cause of

ABBREVIATIONS: IR: Ionizing radiation, PSC: posterior subcapsular cataract, ARC: 1 age-related cataract, A-bomb: atomic bomb, DSBs: double strand breaks, mtDNA: mitochondrial DNA, ATM: ataxia telangiectasia mutated, ATR: ataxia telangiectasia and Rad3-related, BER: base excision repair, NER: nucleotide excision repair, LECs: lens epithelial cells, AGE products: advanced glycation end products, sHSP: small heat shock protein, LMDP: large molecule diffusion pathway, ROS: reactive oxygen species, FGF: fibroblast growth factors, NASCA: NASA Study of Cataract in Astronauts, ICRP: International Commission on Radiological Protection, SSBs: single strand breaks, RIBE: radiation-induced bystander effect, LFCs: lens fiber cells, ND: not determined

cataract and the occupational lens dose limit is reduced from 150 to 20 mGy / year averaged over 5 years (ICRP Publication 118). Understanding the effects of low dose IR on the lens and its role in cataractogenesis is therefore very important. So we redefine “cataractogenic load” as a term to account for the combined lifestyle, genetic and environmental processes that increase biomolecular damage to lens macromolecules. These processes weaken metabolic defenses, increase post-translational protein modifications, and alter the lipid structure and content of the lens. IR exposure is a significant insult to the lens because of free radical generation and the ensuing oxidative stress. We support the concept that damage caused by IR compounds the aging process by increasing the cataractogenic load, hereby accelerating lens aging and its loss of function.

Keywords: Eye lens, cataract, ionizing radiation, posterior subcapsular cataract, aging, double strand breaks, reactive oxygen species, lipid peroxidation

1 INTRODUCTION

Ionizing radiation (IR) exposure induces an iconic eye pathology, namely lens cataract [1, 2]. The lens patho-phenotype typical of a previous IR insult is reported to be a particular type of cataract, namely posterior subcapsular cataract (PSC; [3-5]), but cortical and nuclear cataracts are also reported [4]. PSC is also not unique to IR exposure as it is associated with aging, although nuclear cataract is the most prevalent phenotype of age-related cataract (ARC) [6-9]. A mechanistic explanation has been proposed for nuclear cataract based on an age-related change in lens physiology and a consequential dramatic increase in the oxidation of nuclear lens proteins [10].

Here we reconsider the concept of “cataractogenic load” to explain the process of IR induced cataractogenesis. We redefine the concept as originally proposed [11] to now include damage not only to the epithelial cell genome, but also to all biomolecules in the lens, independent of whether this be caused by IR or age-accumulated modifications to the proteins, lipids and DNA in lens cells. This reflects recent data on protein [12-14] and lipid stability in the lens [15-17], the proposed large molecule diffusion pathway [18] and the accumulation of metabolites, derived from tryptophan [19, 20], glutathione [21], sugars and carbonyl compounds [22, 23] that arise in an age-dependent manner. Both proteins and lipids in the lens accumulate modifications as the individual ages [10, 14, 15] and IR is another potential cause of these protein and lipid modifications. This is in addition to any DNA damage sustained by nucleated lens cells [24]. Here we redefine cataractogenic load

ABBREVIATIONS: IR: Ionizing radiation, PSC: posterior subcapsular cataract, ARC: 2 age-related cataract, A-bomb: atomic bomb, DSBs: double strand breaks, mtDNA: mitochondrial DNA, ATM: ataxia telangiectasia mutated, ATR: ataxia telangiectasia and Rad3-related, BER: base excision repair, NER: nucleotide excision repair, LECs: lens epithelial cells, AGE products: advanced glycation end products, sHSP: small heat shock protein, LMDP: large molecule diffusion pathway, ROS: reactive oxygen species, FGF: fibroblast growth factors, NASCA: NASA Study of Cataract in Astronauts, ICRP: International Commission on Radiological Protection, SSBs: single strand breaks, RIBE: radiation-induced bystander effect, LFCs: lens fiber cells, ND: not determined

as a term to describe the accumulated damage to DNA, proteins and lipids that may collectively contribute to the eventual development of a lens cataract.

We discuss how exposure to low dose IR contributes to the development of lens cataract later in life and why this apparently favours PSC rather than nuclear cataract. Previous studies have not shown significant association between IR exposure and nuclear cataract in later life [2, 5], but a study of the atomic bomb (A-bomb) survivors evidenced that younger people (<30 years; mean age at the time of exposure 10.5 years [4]) were more susceptible to PSC [4, 25] and cortical cataract [4]. Most of the IR study groups, namely the Chernobyl cleanup workers [5], those treated for haemangioma in their early childhood [26] and the Mayak Production Association workers [27] were exposed to IR as juveniles or adults in the third and early fourth decade of life. As the latency for cataract development after a low dose IR insult can involve one to three decades [5,27], it is time to re-examine the connection between IR exposure, aging and the PSC phenotype in order to advance our understanding of the mechanisms involved in low dose IR-induced cataract. In the field of radiation protection, low dose has widely been defined as <0.1 Gy [28], but for the purpose of this review, we define low dose as <0.5 Gy according to the latest threshold for cataracts recently recommended by the International Commission on Radiological Protection (ICRP) [29].

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2 PHYSIOLOGICAL AGING

2.1 *Aging hypotheses and mechanisms*

Normal cell and tissue homeostasis is continually challenged throughout life. To maintain homeostasis, there are many molecular pathways to repair, eliminate and/or replace damaged molecules, cells and tissues [30]. With age, the ability to adequately respond to the environmental and internal stresses is dramatically reduced [31]. Consequently, the damage to nucleotides, proteins and lipids is not properly repaired, and damage accumulates with increasing age [31, 32]. The reduced ability to respond to such stresses, the presence of time-dependent cumulative damage, the loss of homeostasis and resulting increased susceptibility to disease are the “classical” definition of aging [31-33]. The generation of free radicals capable of damaging proteins, lipids and nucleotides [31, 34, 35] was proposed to drive aging and therefore ultimately determine lifespan [36]. This is the oxidative stress hypothesis [37] that later included other free radicals [38] as part of the more general concept that the accumulated damage shortens lifespan [39, 40]. The original free radical concept was later broadened to include the impact of free carbonyls [41] and more recently the possibility that metabolic activity *per se* is a key factor as evidenced by the expansion with age of what has been termed “the deleteriome” [42]. Thus, there is potential for cellular metabolites themselves to drive macromolecular damage in an age-dependent manner [39]. Indeed the inter-relationship between aging and age-related diseases (ARDs) gives credence to the view that aging itself is a disease as the mechanisms that underpin aging also drive ARDs that shorten lifespan [43]. A logical conclusion from such concepts is that ARDs arise via accelerated aging. For the individual, it is both their genetic and environmental background as well as lifestyle that determine the rate of stress adaptation, proteostasis, stem cell exhaustion, metabolic deregulation, macromolecular damage, epigenetic modifications and inflammation which will ultimately determine how quickly a disease threshold for a particular tissue system is reached during an individual’s lifespan [44].

Free radicals can also be produced by external factors (e.g. X-rays); however as they are formed they continuously challenge the integrity and stability of DNA [45], proteins and lipids [46]. This damage is often repaired or countered by cellular defenses. In the case of DNA, there is a complex network of repair mechanisms [47]. The genetic lesions arising include point mutations, translocations, chromosomal aneuploidies, telomere shortening [48, 49]. DNA is the only macromolecule that relies on repair of existing molecules through the whole life of the cells [50]. Unless DNA damage is repaired, the DNA mutations can influence the expression of essential genes and transcriptional pathways, resulting in dysfunctional physiology and accelerated aging [33, 51]. Recognition of DNA double strand breaks (DSBs) involves Ku70/Ku80 or Mre11/Nbs1/Rad50 complexes [50, 52, 53]. The initiation of the downstream repair pathways requires activation of ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR) kinases [54] followed by phosphorylation of histone H2AX [55] and other downstream targets including checkpoint kinases 1 and 2 and ligase IV/XRCC4 complex [56]. Damaged bases and nucleotides are repaired by base excision repair (BER) and nucleotide excision repair (NER) pathways [57, 58]. The genomic stability systems also include specific mechanisms for maintaining the appropriate length and functionality of telomeres and for ensuring the integrity of mitochondrial DNA (mtDNA) [59, 60].

In proteins, oxidative stress causes oxidation of amino acid side chains, fragmentation of polypeptide chains, generation of cross-links and conformational changes. These oxidative modifications are usually irreversible and lead to serious disruption of protein function [61], attracting the attention of protein chaperones [62, 63]. The solution appears to be to continually turnover proteins [63], removing the damaged proteins via the autophagy and/or proteasome pathways [62, 64, 65]. This works for and is sustainable in transcriptionally active cells, but is not the case for denucleated or organelle-free cells such as mature erythrocytes and lens fiber cells (LFCs) where protein damage still occurs. Whilst erythrocytes are replaced regularly, the lens is a closed system that retains all its fiber cells throughout the entire lifetime of the individual.

Oxidative stress also damages lipids [66, 67]. Lipids are important structural components of cell membranes but they can also serve as an energy reserve [68]. Lipid homeostasis and metabolism are key to understanding lifespan and disease [69-71] and are directly influenced by lifestyle [71]. A wide range of diseases involving lipids and their derivatives have been described [72-76] and are also linked to cataract [77-81]. Such observations link lipids, lifespan and the lens.

Generic anti-oxidant and free radical defence systems are available in cells. Anti-oxidants such as glutathione, vitamins C and E as well as enzymes to produce these antioxidants and remove free radicals include superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase [82]. During aging the balance between oxidants and the mechanisms that protect against oxidation changes and the numbers of damaged proteins and lipids increase [31, 32].

2.2 The contribution of genetics to aging

The influence of genetic factors in human longevity is limited to just 20–30% [83] of total aging insults [84-86]. Significant differences in aging and lifespan have been described between monozygotic human twins [87-89], and diet/ lifestyle is but one potential modifier [70, 71, 90]. This still leaves 70–80% of the aging process to non-genetic, stochastic environmental factors. It is well established that these environmental stressors induce epigenetic changes in genetically identical individuals [91-94]. These epigenetic changes due to moderate stress induce expression of genes responsible for stress-resistance delaying the aging process. However, severe stress causes an increase in accumulation of physiological damage and abnormalities, accelerating the process of aging [95, 96].

This means that chronological age and biological aging can differ. The biological aging process involves interaction between genes, proteins and their products, environmental stressors and diet throughout the life of the individual. The combination of internal and external stressors, diet and life style are considered to be the main factors contributing to accelerated biological aging, which results in increased susceptibility to pathologies associated with aging, and decreased longevity [43, 97, 98].

3 LENS BIOLOGY AND ITS AGING FEATURES

The vertebrate ocular lens is an avascular, transparent organ which refracts the incoming light onto the retina in partnership with the cornea. The lens itself comprises a lens capsule, a single layer of lens epithelial cells (LECs) covering the anterior half of the lens (Figure 1)

and lastly the LFCs, which differentiate from LECs are produced in the germinative zone at the lens equator (reviewed in [99]). The development and maintenance of optical transparency, refraction and elasticity is crucial for the physiological function of the lens. All change as a result of aging, with presbyopia (gradual deterioration of the ability to focus on close objects) being the universal phenotype experienced by everyone who reaches their fifth decade of life [100]. Presbyopia represents a major functional compromise of age-dependent lens function (Figure 2). Importantly, the lens is a closed system [101] where proteins and lipids are retained from lens formation during development in utero to the point of death.

To ensure lasting lens transparency, maturing LFCs undergo complete loss of organelles such as the cell nucleus, mitochondria and endoplasmic reticulum [102] and accumulate a remarkably high concentration (up to 600 mg/ml) of crystallin proteins which not only form the lens refractive index gradient, but also provide the chaperone complement of the lens (reviewed in [102]). Due to the absence of organelles in mature lens fibers, the need for nutrients, water, and ions is satisfied via a unique lens circulation system [103]. During fiber cell elongation, cells undergo extensive cytoskeletal remodelling (reviewed in [99]) and establish cell to cell communications through the expression of the water channel aquaporin 0 (AQP0) and the connexins Cx50, Cx46 and Cx43 that form gap junctions [100, 104].

To aid lifelong transparency and optical function, the lens also utilises a regulation system to protect itself against oxidative stress [105-108] on both a proteostatic level and via age-related changes in the membrane lipid composition to reduce oxygen levels in the lens nucleus [109, 110]. The main anti-oxidant enzymes are superoxide dismutase, catalase, glutathione redox cycle enzymes and peroxiredoxins. Superoxide dismutase converts superoxide radicals into oxygen and hydrogen peroxide. Catalase, glutathione redox cycle enzymes and peroxiredoxins collaborate to convert hydrogen peroxide into water molecules [111]. Reduced glutathione is newly synthesized in the lens epithelium and outer cortical fiber cells [112], and functions as the prime anti-oxidant agent by diffusing to the lens nucleus through gap junctions [113].

Tissues like the lens [10] and the erythrocyte [114] are valuable models of aging as they reveal key biological principles in a cellular background partially independent of genetic regulation. As most of the cells comprising the eye lens lack nuclei and biosynthetic organelles (reviewed in [99]), it is an excellent model to study the impact of retained macromolecular damage on aging. Long lived proteins are a feature of many cells and tissues, their turnover being related to both their structure-function role and to their cell environment [115]. Indeed, the lens relies on particularly long-lived, abundant proteins (e.g., crystallins) for its optical clarity and function [12, 13, 63]. Therefore, preservation of homeostasis is performed in a protein environment which accumulates post-translational modifications as a function of age [10].

3.1 The importance of aging in cataractogenesis

As the lens is a closed system [101] where protein turnover is very limited, it is a tissue where most of its protein [12, 13] and lipids [15-17] are as old as the lens itself. As a point in case, proteins in the lenses of the Greenland shark are retained for at least 272 years [116]. Aging of the lens macromolecules is therefore within a unique cellular environment and whilst lens function is maintained, these macromolecules are continually being

compromised. For example, after 5 decades, lens stiffness increases so that near vision is compromised [117, 118]. Subsequently ARC develops with three possible phenotypes – nuclear, posterior subcapsular and cortical cataract [6, 7, 119, 120]. Of these, nuclear cataract is the most prevalent (up to 50%), although PSC still represents a significant proportion among certain population groups (~11%; [7, 121]). Cortical cataract had a higher incidence in younger people (<50 years old; [6, 119]), suggesting that the cortex of the lens could be more vulnerable in an aging context. As the individual ages, the lens changes too. The latency for ARC by definition is decades and coincidentally the appearance of PSC/cortical cataract after exposure to low dose IR as reported for A-bomb survivors can also be decades, although PSC rather than nuclear cataract is more frequent [4]. These observations require further investigation to connect cataract phenotype, age of incidence, and particular environmental stressors.

3.2 Contribution of aging to cataract formation

Genetics, lifestyle and environmental factors all influence aging of the individual [122] and it has proven difficult to capture this within a single theory. Previous theories of aging have included the programmed theory [123], the evolutionary theory of aging with its key concepts of mutation accumulation and antagonistic pleiotropy as proposed by Medawar and Williams respectively, the free radical theory of aging [36], the disposable soma theory [124] and lastly the hyperfunction theory of aging [125]. The recent proposal of a “deleterome” accounts for the varied contribution of genetics and environment in the aging process and incorporates aspects from all previous theories [122]. We propose “cataractogenic load” as the equivalent for the lens and the process of cataractogenesis (Figure 2), given that the lens is a tissue where biomolecular longevity (Stewart et al 2013; Hughes et al 2016), lifelong retention of lens components and continued growth are integral to lenticular homeostasis [118]. The genetic and environmental influences upon cataractogenesis are well documented [126]. The phenotypic heterogeneity between families or even between family members with the same autosomal dominant mutation evidences multiple mechanistic influences upon the cataract phenotype [126]. As the lens ages so its metabolism changes too, not only in its response to growth factors [127], but also as the result of the buildup of metabolites derived from tryptophan breakdown to form kynurenine derivatives [19, 128], glutathione breakdown [21] as well as the increase in advanced glycation end (AGE) products [22, 23]. These metabolites are all capable of post-translational modifying lens proteins and this is in addition to the racemization/isomerization events that occur in long lived proteins including lens proteins [14].

The term “cataractogenic load” acknowledges this complexity of the lens aging process and encapsulates the importance of accumulated damage to nucleotides, proteins and lipids [10, 24, 129] and the metabolic changes that contribute to the lens deleterome as contributory mechanisms in cataractogenesis. This is especially relevant to the development of ARC [6, 7, 119, 120] and low dose IR-induced cataract ([29]; Figures 2 and 3), both requiring long latency periods.

3.2.1 Age-related changes in the lens epithelium, its genome and exome

The logarithmic increase in lens size continues until birth and shortly afterwards lens growth becomes linear in humans [101]. The underlying mechanism for this sudden decrease in cell proliferation is not fully understood. Fibroblast growth factors (FGF) levels, cell proliferation and cell density in the central and germinative zones decrease in

an age-dependent manner [127, 130]. Similar changes in epithelial cell density are seen in other mammals [131, 132] and primates [133]. Some have proposed that increased oxygen levels also contribute with increasing age [134]. The integrity of the meridional rows deteriorates with age, becoming more disorganized in older humans [131]. Coincidentally, similar changes occur after IR exposure (15 Gy) of lenses in young animals [135].

In addition to these gross changes in epithelial cell density, both DNA damage [136] and an age-dependent decrease in the expression levels of DNA repair genes occur in LECs [137, 138]. The resulting accumulation of DNA damage is thought to be associated with the development of ARC [24]. Indeed, changes induced by ultraviolet B (UVB) exposure can repress the DNA repair gene *ERCC6* via hypermethylation [139]. Telomeres are also shortened in cataractous lens samples [140], suggesting a link between ARC and an increase in oxidative stress [141] and perhaps also a link with senescence [142]. Lifelong exposure to UV light is associated with accumulated oxidative stress and is thought to be a factor in ARC [19, 20, 143]. Oxidative stress triggered DNA damage requires repair by BER and NER pathways [144]. In addition to known risk factors in the development of ARC [145-149], there are also positive lifestyle influences that delay its development, e.g. exercise [147] and diet [150], but the phenotype of both ARC and congenital cataract will be subject to the influence of genetic, metabolic and environmental factors [126].

3.2.2 Aging of lens proteins

The lens contains the highest concentration of small heat shock protein (sHSP) chaperones (α -crystallins) among all mammalian tissues [151, 152]. Their role is to prevent protein aggregation, primarily of other crystallins [153]. The lens contains some of the oldest proteins in our bodies [12]. A large molecule diffusion pathway (LMDP) operates in the lens [18]. An exchange of proteins in the soluble fraction between the oldest (central; nuclear) and youngest (peripheral; cortical) cells and vice versa in the lens is suggested from these data [13] possibly as an LMDP [18] component and despite the development of lens barrier in later life [112]. Transfer of some of the proteostatic load accumulating in the oldest lens cells to those with better proteostatic [154] and biosynthetic capacity [155, 156] would be advantageous as suggested from the latest analysis of protein age in the lens [13]. The proteasome [154, 157, 158], calpain [159] and autophagy [160] systems all contribute to proteostasis in the lens.

As lens proteins are long-lived, some amino acids spontaneously decompose (e.g. racemisation; deamidation [14]) due to their intrinsic instability. Although they are also modified by cellular metabolites, this is an order of magnitude less than racemisation and deamidation [10]. To put this in perspective, the proteins in old human lenses have a very low AGE content [10]. Deamidation on the other hand can correlate with increased insolubility [161], reduced chaperone surveillance [162] and with ARC [162].

Damage to the most prevalent proteins in the eye lens, crystallins, is mainly caused by oxidative stress [163]. Reactive oxygen species (ROS) react with crystallins and other lens proteins (Figure 3), contributing to the age-dependent increase in post-translationally modified proteins [163, 164]. Accumulation of these modifications results in protein aggregation (Figure 3), precipitation and eventually the loss of lens transparency and function [165]. At the start of the fifth decade of life, there are significant changes in the lens proteome, the membrane association of previously soluble lens proteins, and an increase in lens stiffness, all of which coincide with the development of presbyopia ([117]; Figure 2).

At the same time, investigation into the correlation between aging and the glutathione gradient in the eye lens showed that glutathione diffusion from the cortex into the nucleus is impaired in older human lenses compared to young lenses [112]. A barrier develops effectively isolating the nucleus of the lens and compromising the reductive defence system of this region [117]. This is one of the paradoxes regarding glutathione homeostasis in the lens [107] as it is unexplained why small anti-oxidants like glutathione are unable to diffuse freely into the lens nucleus, whilst proteins appear capable of slow exchange [13, 18].

Overall the levels of superoxide dismutase, glutathione redox cycle enzymes and peroxiredoxins have been shown to decrease with age, which allows ROS to accumulate in the lens [111, 166]. This is despite the fact that the lens has a well-developed redox regulation system to protect itself against oxidative stress [108]. Also, age-related changes in the membrane lipid composition reduce oxygen levels in the lens nucleus [109, 110].

3.2.3 Age-related changes in lens lipids

Another vital component for the maintenance of lens transparency and preventing oxidative stress induced damage in the lens nucleus is the lens lipidome. The majority of the phospholipids in human lens membranes are phosphatidylethanolamine, sphingomyelin, and dihydrosphingomyelin, of which dihydrosphingomyelin is the most abundant [167]. The human lens has the highest cholesterol concentration among all tissues [168]. With age, the levels of sphingomyelin and dihydrosphingomyelin increase while the levels of glycerolipids such as phosphatidylethanolamine decrease [167]. Ceramides increase dramatically in people older than 30 years of age, whilst glycerophospholipids (with the exception of lysophosphatidylethanolamines) decline rapidly and are almost depleted in people aged 40 years [16]. Cholesterol levels also increase with age [169]. Glycerolipids are less saturated than dihydrosphingomyelin and therefore more sensitive to oxidative stress [170]. Besides unsaturated glycerolipids, cholesterol is oxidized and its levels correlate with ARC [129], again indicating how IR-induced ROS can also potentially modify the lens lipidome (Figure 3). Oxidized lipids trigger changes in the membrane lipid interactions, which lead to disruptions in membrane structure [171]. These disruptions are recognized by phospholipase A2 and removed [172]. It has been hypothesized that the degraded oxidized lipids are replaced by dihydrosphingomyelin and cholesterol, which results in more rigid membranes and increases light scattering [167, 173, 174]. Sphingomyelins are long lived and appear not to turnover in the lens nucleus [17]. Sphingomyelins are thought to increase resistance to oxidation in the lens nucleus of long-lived mammals due to their stable, saturated side chains [109]. Equally, cholesterol domains are also important in reducing oxygen diffusion in the lens [175].

Changes in membrane composition affect interactions with proteins and vice versa [176]. AQP0 is the most abundant protein in lens membranes, and its structure and function are lipid-dependent [177]. The increase in cholesterol and sphingomyelin has been shown to cause a decrease in the permeability of AQP0 water channels [178]. The binding of α -crystallin to the membrane also decreases with increasing cholesterol levels [179]. How all these age-related changes in lipid composition affect AQP0 and α -crystallin binding remains to be elucidated. Nevertheless, there is a correlation between the accumulation of certain oxidized cholesterol and ARC [129] evidencing that changes in the lipidome of lens membranes accompanies cataractogenesis. Oxysterols affect the stability of lenticular protein chaperones [180] and can reverse ARC in some animal models [181], but they also activate small GTPases with consequential changes in the lenticular cytoskeleton [182] and

gap junction-mediated intercellular communication [183]. Lipids are therefore active modulators of lens cell physiology.

3.3 *Cataract is primarily an age-related disease*

Cataract is one of the main causes of blindness around the world. The age of onset is usually used to classify cataract [126, 184]. Although congenital, juvenile and pre-senile (before the age of 45) cataracts are observed [185, 186], ARCs or senile cataracts are the most prevalent type of cataract with population studies indicating an ARC incidence up to 85% of the cataract affected cohort [187]. The Beaver Dam Eye Study, involving 4926 individuals between the age of 43 and 84 years, demonstrates a direct correlation between cataract incidence and increasing age [7, 188]. Congenital cataracts tend to be caused by mutations in genes coding for proteins vital for lens structure and membrane transport including crystallins [126]. Although genetics have been implicated in ARC pathogenesis [189, 190], lifestyle choices such as smoking [191], alcohol consumption [192] and the individuals environment specific circumstance (e.g. exposure to sun light [19, 193, 194]) are also key factors affecting the age of onset. A study on the involvement of genetics in congenital cataracts and ARC suggests that a limited number of genes have a direct association with increased ARC risk and that these gene mutations result in pleiotropic ARC subtypes [190, 195]. Major genes identified are GALK1, EPHA2, and CRYAA, though others such as GSTM1, and the DNA repair genes WRN and XPD show inconsistent association and penetrance [190, 195-199].

4 THE LINK BETWEEN IR EXPOSURE AND AGING

4.1 *Exposure to high IR doses leads to presenile cataract*

IR is an important environmental factor in cataract development and the etiology of many other diseases. The effect of high dose IR on human physiology has been widely studied [200-203]. The biological response to high IR dose includes oxidative stress, senescence, and activation of genes linked to aging [204, 205], indicating that high IR doses are a premature age stressor. Research on the physiological, cellular and molecular responses of the eye lens to IR has been ongoing since the discovery of X-rays in 1895 [206] and the associated cataract phenotype [207]. Early studies of IR-induced cataracts that developed in Hiroshima and Nagasaki A-bomb survivors after 6–30 months [3, 208, 209] noted the formation of a central opacity that could adopt a doughnut-like appearance with a diameter of 3–4 mm [209]. Granular opacities and vacuoles sometimes also appeared in the anterior subcapsular region. The initial opacities also had a yellowish (bronzed) hue [209]. The age range of these survivors, who were within a 1000 m of the hypocenter, was between 13 and 50 years. As all the other individuals within this zone died from radiation sickness, shielding by inanimate objects secured survivors. This indicates high dose IR-induced cataracts have a short latency period and lead to presenile cataract [209]. A-bomb survivor studies also considered whether the age of the individual affected the appearance of axial and PSC opacities. For older individuals (>70 years), there was a negative correlation with age for axial opacities, suggesting that the risk was minimal for this cataract type, however a positive correlation was observed for PSC [25, 210] and cortical cataract [4]. Limited histological analysis of IR-induced cataract were gathered from A-bomb survivors, cyclotron workers and those undergoing radiotherapy for a range of ocular tumors and conditions including retinoblastoma [208]. These revealed a significant disorganization and multicellularity of the lens epithelium particularly in the equatorial region, the loss of

meridional row organization and a breakdown in the lens epithelial layer compartmentalization. Similar histopathological changes are observed in other acute insults such as steroid treatment [211, 212] noting that PSC can be caused by a variety of methods and medical conditions [121]. These observations and the transient nature of some PSCs [213-215] are evidence to suggest that the cortex is more sensitive to acute changes in environment and physiology than the lens nucleus.

4.2 *Acute versus protracted or chronic IR exposure and lens cataract*

Whilst A-bomb survivors received acute doses, people are usually subjected to low dose protracted or chronic exposures. For instance, aircraft crew, cardiologists and radiologists all receive occupational exposure to protracted IR doses. After the Chernobyl nuclear power plant accident, the protractedly exposed liquidators were followed up and the incidence of PSC and cortical cataract reached 25% 14 years post-exposure to doses up to 1 Gy [5]. A comparison of cataracts in astronauts and military aviators showed higher cataract incidence in astronauts [216]. The cataracts of military aviators were mainly PSC, while astronauts were more often diagnosed with cortical cataracts. In line with the latter, the NASA Study of Cataract in Astronauts (NASCA) reported an increase in both median and variability of cortical cataract compared to non-exposed astronauts, and identified a relationship between cumulative galactic cosmic radiation dose and cortical cataract [217, 218]. Confirming to Mark Little's review recognizing the absence of association between nuclear cataracts and IR [2], these observations support the concept that the lens cortex is more vulnerable to IR damage compared to the nucleus. Also, IR exposure of ground-based workers, even at low dose, correlates with pre-senile cataractogenesis as compared with age-matched controls. For instance, a Polish interventional cardiologist cohort reported a range of 0.4–1.55 Gy [219], whereas retrospective studies on radiologic technologists in the US estimated a mean dose of 77 mGy in a cohort of 109,300 in 2014 [220] and more recently in 2018, the mean cumulative estimated 5-year lagged eye-lens absorbed dose associated with self-reported cataract was 55.7 mGy [221]. Dose exposure calculated from self-reported workloads shows a wider range, with a cardiology conference cohort presenting a range of 0.1–18.9 Gy [222]. The reported incidence of lens opacities in exposed medical personnel varies both in the intensity and region of the lens affected. PSCs have been reported in 17% of the exposed O'CLOC cohort as opposed to 5% of controls [223] and in 50% of cardiologists and 41% of nurses and technicians in comparison with <10% of age-matched controls in a cardiology congress cohort [222]. A follow up study of 13,902 childhood cancer survivors who received radiation therapy as part of their treatment showed a strong correlation between 0.5 and 1.5 Gy dose exposures and the development of pre-senile cataracts later in life [224]. Therefore exposure to IR increases the risk of PSC [225, 226], but other ARC phenotypes including cortical and nuclear cataract [3, 4, 6, 7, 119, 120, 210, 227] are also observed.

Based on these findings, ICRP revised previous guidance [228] and suggested a nominal threshold of 0.5 Gy for cataract formation independent of the rate of dose delivery [29]. Current ICRP recommendations are for a reduced occupational lens dose limit of 20 mGy year⁻¹ (averaged over 5 years with no single year >50 mGy), highlighting the importance of determining in detail the biological response of the eye lens when exposed to low dose IR given its importance to present and future ICRP recommendations. Moreover, the US National Council on Radiation Protection and Measurements (NCRP) notes the urgency for lens-specific dosimetry and that until those technical challenges are optimised, lens-exposure should be regarded similar to whole body exposure [229-231].

4.3 Lower doses are correlated with a longer latency period

The mechanisms underlying IR-induced cataract are not fully understood and more research is ongoing to refine the proposed nominal threshold of 0.5 Gy [29]. In considering the lens cataract phenotype, the key variables that will influence the timing and phenotype of the IR-induced cataract will be the IR dose administered and the age of the individual. Early studies investigated acute IR doses (10's of Gy) that induced cataract within weeks or months in both animals [135, 232] and humans [3, 208, 209]. These early studies also identified that the age of animal at the time of exposure influenced the latency of the acute phenotype [232] and this was later interpreted as further evidence that the lens epithelium, and specifically the proliferating cells at the lens equator were the primary target for acute IR exposure [11]. As the latency for development of some cataracts in A-bomb survivors exceeded even half century [4], reassessment of the relation between IR exposure, aging and the PSC phenotype will provide us with a deeper insight of the biological mechanisms underlying low dose IR induced cataracts.

5 EFFECTS OF IR ON THE LENS

Details of the biochemical and cell biological changes in IR-irradiated human lenses are sparse (Table 1) and limited to gross phenotypic changes, such as vacuoles and cataracts formed in the posterior subcapsular region [3, 26, 207-209] due to the disruptive nature of corrective surgery and the availability of human tissue. In the following section we discuss IR-induced damages and which processes have been shown to manifest in lens cells.

	Aging	IR > 0.5 Gy	IR < 0.5 Gy
Gene expression	✓	✓	✓
DNA repair	✓	✓	✓
Cell density	✓	✓	✓
Proliferation	✓	✓	✓
Integrity of meridional rows	✓	✓	ND
Deamidation	✓	✓	ND
Racemisation	✓	✓	ND
Truncation	✓	✓	ND
Methionine oxidation	✓	✓	ND
Increased disulphide bonds	✓	✓	ND
Increased sphingomyelin and dihydrosphingomyelin	✓	ND	ND

Decreased glycerolipids	✓	ND	ND
Advanced glycation end products	✓	ND	ND
Kynurenines	✓	ND	ND
Cholesterol oxidation	✓	ND	ND
Increased cholesterol	✓	ND	ND
Cholesterol domain formation	✓	ND	ND

Table 1: Overview of the biochemical and cell biological effects of aging and IR exposure on the lens and lens cells. The biological mechanisms underlying ionizing radiation (IR) - induced damage to the eye lens are not completely understood. A survey of the literature shows that aging and IR > 0.5 Gy cause similar damage to the eye lens, while our knowledge of the effects of IR < 0.5 Gy is quite limited. Future research will elucidate whether the aging and IR > 0.5 Gy and < 0.5 Gy have similar effects on the eye lens, and therefore whether IR leads to accelerated aging. ND: not determined

5.1 DNA damage induced by IR exposure

IR induces direct DNA damage by generating physical breaks in DNA structures, and indirect damage through interaction with molecules leading to the formation of free radicals [233]. Direct and indirect damage include the production of single strand breaks (SSBs) and DSBs in DNA. IR-induced SSBs can be efficiently repaired [234]. In contrast, DSBs have been shown to be the main source of IR-induced mutations [235].

Genotoxicity has been observed in non-irradiated portions of the body resulting from IR exposure of other areas. First reported in 1954, where irradiation between 7.36 and 28.41 Gy directed to the spleen produced damage in bone marrow cells [236], this phenomenon is known as the abscopal effect [237, 238]. It is caused by irradiated tissue releasing molecules, e.g. ROS, that are transferred to other non-irradiated tissues, where they induce similar deleterious effects [239]. Though not directly demonstrated in the lens, the abscopal effect poses an interesting possibility in the overarching issue of eye health. Another, more recently described, consequence of indirect DNA damage is radiation-induced bystander effect (RIBE) wherein non-irradiated cells show similar features as irradiated cells as a result of intercellular signalling [238, 240]. The mechanisms underlying RIBE in other organs, e.g. transport of free radicals and ROS via gap junctions, are processes that also take place in the eye lens [241], which suggests that the occurrence of RIBE in the eye lens is possible [242].

Genomic instability is another downstream effect of IR. During proliferation of cells containing IR-induced mutations, these DNA alterations can be transferred to their daughter cells. Signals leading to production of intracellular ROS is transmitted to daughter cells, where they can cause additional DNA damage [243].

5.2 IR induced damage in the lens epithelium – Aberrant proliferation and differentiation

Our mechanistic understanding of IR-induced cataract is largely informed by animal models, but collectively with human studies they evidence a significant role for the lens epithelium in IR-induced cataract [11, 244]. High dose IR (15 Gy) caused a decrease in cell density in the germinative zone of rabbit lenses in the short term followed by a proliferative burst before returning to a pre-exposure baseline several weeks later [135]. Repressing LEC proliferation prior to IR-exposure (6 Gy/min) produced resistance to damage in a frog model suggesting cell proliferation was a key process involved in IR-induced cataract [244]. *In vitro* experiments with HLEC1 cell line showed that irradiation with ≥ 2 Gy (but not at < 2 Gy) stimulates the proliferation of human LECs [245]. Besides aberrant cell proliferation and epithelial organization, epithelial differentiation was also changed after IR exposure (2–20 Gy; [11, 246]). These experiments were all performed with high-dose exposures. Markiewicz and colleagues performed *in vitro* and *in vivo* irradiation experiments, with the FHL124 cell line and mice respectively and included besides high dose also low dose exposures (20 mGy – 2 Gy; [247]); dose dependency in DSBs as phosphorylated histone H2AX (γ H2AX) foci, increased proliferation and cell density at doses < 0.5 Gy were still observed [247]. Changes in proliferation and altered gene expression were seen in the HLEC1 cell line at 4 Gy IR exposures, but not at 0.5 Gy [248] questioning whether the observed cell density and proliferation changes at low dose IR require altered gene expression.

5.3 Altered transcription as a result of IR induced DNA damage and repair

Transcriptionally active genes in cancer cells have been shown to be prone to mutation [249]. Although DNA DSBs are introduced randomly into the genome after IR exposure, those genes most likely to retain mutations are transcriptionally active genes [249]. Mutation rates in exons are lower than those found in introns because of differential mismatch repair in exons and introns [250] and H3K9me3, as a marker for open, transcriptionally active chromatin regions, is associated with higher mutation rates [251]. Recent evidence suggests that DNA repair processes involve the expansion of chromatin and eviction of histones at the site of the DSBs [252], in support of differences in DNA repair efficiency between transcriptionally active and inactive chromatin regions. For the lens this would mean that genes such as the crystallins would be more susceptible [253]. The LECs at the lens periphery have been shown to repair DSBs more slowly [247] and along with the nucleated, differentiating LFCs these are the lens cells that are transcriptionally active. Moreover, crystallin gene transcription is dependent upon both p53 [254] and N-myc [255], providing another potential direct link between transcription and DNA repair [256]. Given the long latency between exposure to low dose IR and the appearance of cataract, this is a potential mechanism contributing to the low dose IR-induced phenotype.

5.4 Reduced functionality of DNA repair pathways in lens cells

Exposure of rabbit lens epithelium to UVA (180 kJ/m²) showed that irradiation leads to SSBs [234]. The amount of SSBs was positively correlated with exposure dose. However, these did not cause opacities because 80% of the SSBs were repaired within a timeframe of 4 hours. The effect of low dose IR on the activity of DNA repair mechanisms was measured using DSB markers γ H2AX and RAD51 (ranging from 20 mGy to 2 Gy; [247, 257]). γ H2AX codes for an activator of DNA repair and RAD51 is part of the homologous recombination DNA repair systems. In FHL124 cells, the expression levels of these markers

showed a linear dose response to exposures at lower than 0.5 Gy. Mouse LECs exposed to 0.01 and 0.1 Gy showed an increase of γ H2AX. This increase was higher in the central zone than in the germinative and transitional zones. Comparison of γ H2AX levels in the LECs with those in lymphocytes showed that DNA repair in LECs is slower [247]. Based on the latter, we conclude that irradiation leads to a slower DNA repair in the LECs. In particular, DNA repair appears to vary between lens epithelium zones with the transcriptionally active germinative and transitional zones demonstrating slower and fewer repair incidents [135, 247].

5.5 Effects of irradiation on the fiber cell proteins

In order to develop and maintain lens transparency, lens proteins must not aggregate at high concentrations [258]. IR-induced oxidative stress causes aberrant protein aggregation, which leads to light scattering in the lens and eventually leading to cataract formation [259]. These IR-induced aberrant protein aggregates are formed through post-translational modifications including oxidation, deamidation, truncations and cross-linking [111]. Irradiated α -crystallins form aggregates *in vitro* [260]. Follow up studies using γ -irradiated rat lenses showed that this triggered oxidation and deamination of crystallins [261]. Exposure of α -crystallin to UV also resulted in tryptophan degradation and inactivation of their chaperone function [260]. Still, the effects of low dose irradiation on protein structure remain largely unknown [262].

5.6 Effects of irradiation on lens membrane lipids

Cataract patients have increased levels of lipid peroxidation products in comparison to age matched controls [129]. Lipid peroxidation has been shown to cause changes in the structure and permeability of membranes after exposure *in vitro* [263]. IR exposure stimulates formation of ROS which can oxidise the lipids in the eye lens. Oxidation of cholesterol generates oxysterols [264, 265]. However, the mechanisms through which low-dose IR-induced lipid oxidation of cholesterol causes lens damage are now under investigation.

6 CONCLUSION - THE LENS AS A MODEL TO STUDY THE LINK BETWEEN AGING AND LOW DOSE IR

In the lens, the aging process manifests itself first by the development of presbyopia in the fifth decade of life (Figure 2; [117, 118]) and then ultimately in the formation of ARC [10]. These are quite distinct albeit interrelated phenotypic stages [266] and consistent with the concept that ARC represents a disease associated with accelerated aging [43, 44]. Biomolecular damage accumulates during the lifetime of the individual. IR exposure is an addition to the damage accumulated due to the aging process alone (see Table 1). The lens retains lipids [17] and proteins [10] over its lifetime meaning that any IR-mediated non-DNA damage persists and is not removed. Thus, the rate of damage accrual can influence the timing of cataract during the aging process [44] as the cataractogenic threshold is reached sooner (Figure 2). For each individual, this threshold is determined by age, lifestyle, environment and genetic background i.e. all those factors known to contribute to human cataractogenesis from epidemiological studies. The damaging effects upon cells and their biomolecules through the generation of free radicals by IR exposure will be retained in the protein and lipid components in the LECs and LFCs ([10, 24, 129]; Figure 3). DNA damage, though mostly repaired, might also influence lens health via the retention of

mutations or alteration in cell metabolism [33, 51]. This IR-induced damage therefore contributes to the cataractogenic load (i.e. lens deleteriome [122]). The genetic background, metabolic, environmental and lifestyle experiences of the individual will affect both the timing and nature of their cataract (cortical or nuclear) depending on when the cataractogenic threshold is reached (Figure 2).

The lens therefore provides an experimental system to study aging processes. Incidental IR exposure can be measured and experimental doses can be controlled, which is not easy for other environmental insults such as smoking and UV-exposure when linked to cataract [191, 267, 268] and accelerated aging (e.g. [269-272]). The fact that in several cohorts, PSC caused by exposure to low dose IR follows a linear dose response relationship (rather than a threshold-type relationship) for various age groups provides an opportunity to identify the IR-specific cataractogenic load and the mechanism(s) involved. Diverse patient groups have been shown to be key to understanding disease dynamics, with the Beaver Dam Eye Study being a hallmark example of the above [7, 187, 188]. The study of low dose IR and its effect on cataractogenic load can help deliver mechanistic insight into both cataractogenesis and the radiobiological effects of IR. Understanding factors which contribute to increased rate of cataract progression could aid the delay of cataract onset over the lifetime of an individual.

7 ACKNOWLEDGMENTS:

The authors are part of the LDLensRad project that has received funding from the Euratom research and training programme 2014-2018 in the framework of the CONCERT [grant agreement No 662287]. The financial support of the Royal Society (RAQ; IE151271) the National Eye Research Foundation (AAK; SAC014) and Fight for Sight UK (RAQ, MJ; 1584/1585) are also gratefully acknowledged.

8 REFERENCES

- [1] E.A. Ainsbury, S. Barnard, S. Bright, C. Dalke, M. Jarrin, S. Kunze, R. Tanner, J.R. Dynlacht, R.A. Quinlan, J. Graw, M. Kadhim, N. Hamada, Ionizing radiation induced cataracts: Recent biological and mechanistic developments and perspectives for future research, *Mutation research*, 770 (2016) 238-261.
- [2] M.P. Little, A review of non-cancer effects, especially circulatory and ocular diseases, *Radiation and environmental biophysics*, 52 (2013) 435-449.
- [3] R.J. Miller, T. Fujino, M.D. Nefzger, Lens findings in Atomic bomb survivors. A review of major ophthalmic surveys at the atomic Bomb Casualty Commission (1949-1962), *Archives of ophthalmology* (Chicago, Ill. : 1960), 78 (1967) 697-704.
- [4] E. Nakashima, K. Neriishi, A. Minamoto, A reanalysis of atomic-bomb cataract data, 2000-2002: a threshold analysis, *Health physics*, 90 (2006) 154-160.
- [5] B.V. Worgul, Y.I. Kundiyeu, N.M. Sergiyenko, V.V. Chumak, P.M. Vitte, C. Medvedovsky, E.V. Bakhanova, A.K. Junk, O.Y. Kyrychenko, N.V. Musijachenko, S.A. Shylo, O.P. Vitte, S. Xu, X. Xue, R.E. Shore, Cataracts among Chernobyl clean-up workers: implications regarding permissible eye exposures, *Radiation research*, 167 (2007) 233-243.
- [6] G.L. Kanthan, J.J. Wang, E. Rochtchina, A.G. Tan, A. Lee, E.M. Chia, P. Mitchell, Ten-year incidence of age-related cataract and cataract surgery in an older Australian population. The Blue Mountains Eye Study, *Ophthalmology*, 115 (2008) 808-814.e801.
- [7] B.E. Klein, R. Klein, K.E. Lee, R.E. Gangnon, Incidence of age-related cataract over a 15-year interval the Beaver Dam Eye Study, *Ophthalmology*, 115 (2008) 477-482.
- [8] K.E. Lee, B.E. Klein, R. Klein, T.Y. Wong, Changes in refraction over 10 years in an adult population: the Beaver Dam Eye study, *Investigative ophthalmology & visual science*, 43 (2002) 2566-2571.
- [9] P. Mitchell, R.G. Cumming, K. Attebo, J. Panchapakesan, Prevalence of cataract in Australia: the Blue Mountains eye study, *Ophthalmology*, 104 (1997) 581-588.
- [10] R.J. Truscott, M.G. Friedrich, The etiology of human age-related cataract. Proteins don't last forever, *Biochimica et biophysica acta*, 1860 (2016) 192-198.
- [11] B.V. Worgul, G.R. Merriam, Jr., C. Medvedovsky, Cortical cataract development--an expression of primary damage to the lens epithelium, *Lens and eye toxicity research*, 6 (1989) 559-571.
- [12] N. Lynnerup, H. Kjeldsen, S. Heegaard, C. Jacobsen, J. Heinemeier, Radiocarbon dating of the human eye lens crystallines reveal proteins without carbon turnover throughout life, *PloS one*, 3 (2008) e1529.

- [13] D.N. Stewart, J. Lango, K.P. Nambiar, M.J. Falso, P.G. FitzGerald, D.M. Rocke, B.D. Hammock, B.A. Buchholz, Carbon turnover in the water-soluble protein of the adult human lens, *Molecular vision*, 19 (2013) 463-475.
- [14] N. Fujii, T. Takata, N. Fujii, K. Aki, H. Sakaue, D-Amino acids in protein: The mirror of life as a molecular index of aging, *Biochimica et biophysica acta*, (2018).
- [15] D. Borchman, M.C. Yappert, Lipids and the ocular lens, *Journal of lipid research*, 51 (2010) 2473-2488.
- [16] J.R. Hughes, J.M. Deeley, S.J. Blanksby, F. Leisch, S.R. Ellis, R.J. Truscott, T.W. Mitchell, Instability of the cellular lipidome with age, *Age (Dordrecht, Netherlands)*, 34 (2012) 935-947.
- [17] J.R. Hughes, V.A. Levchenko, S.J. Blanksby, T.W. Mitchell, A. Williams, R.J. Truscott, No turnover in lens lipids for the entire human lifespan, *eLife*, 4 (2015).
- [18] Y. Shi, K. Barton, A. De Maria, J.M. Petrash, A. Shiels, S. Bassnett, The stratified syncytium of the vertebrate lens, *Journal of cell science*, 122 (2009) 1607-1615.
- [19] M. Linetsky, C.T. Raghavan, K. Johar, X. Fan, V.M. Monnier, A.R. Vasavada, R.H. Nagaraj, UVA light-excited kynurenines oxidize ascorbate and modify lens proteins through the formation of advanced glycation end products: implications for human lens aging and cataract formation, *The Journal of biological chemistry*, 289 (2014) 17111-17123.
- [20] J. Mizdrak, P.G. Hains, R.J. Truscott, J.F. Jamie, M.J. Davies, Tryptophan-derived ultraviolet filter compounds covalently bound to lens proteins are photosensitizers of oxidative damage, *Free radical biology & medicine*, 44 (2008) 1108-1119.
- [21] M.G. Friedrich, Z. Wang, K.L. Schey, R.J.W. Truscott, DehydroalanylGly, a new post translational modification resulting from the breakdown of glutathione, *Biochimica et biophysica acta*, 1862 (2018) 907-913.
- [22] R.H. Nagaraj, M. Linetsky, A.W. Stitt, The pathogenic role of Maillard reaction in the aging eye, *Amino acids*, 42 (2012) 1205-1220.
- [23] R. Cheng, B. Lin, B.J. Ortwerth, Rate of formation of AGEs during ascorbate glycation and during aging in human lens tissue, *Biochimica et biophysica acta*, 1587 (2002) 65-74.
- [24] K. Sorte, P. Sune, A. Bhake, V.B. Shivkumar, N. Gangane, A. Basak, Quantitative assessment of DNA damage directly in lens epithelial cells from senile cataract patients, *Molecular vision*, 17 (2011) 1-6.
- [25] M. Otake, S.C. Finch, K. Choshi, I. Takaku, H. Mishima, T. Takase, Radiation-related ophthalmological changes and aging among Hiroshima and Nagasaki A-bomb survivors: a reanalysis, *Radiation research*, 131 (1992) 315-324.

- [26] G. Wilde, J. Sjostrand, A clinical study of radiation cataract formation in adult life following gamma irradiation of the lens in early childhood, *The British journal of ophthalmology*, 81 (1997) 261-266.
- [27] T.V. Azizova, E.V. Bragin, N. Hamada, M.V. Bannikova, Risk of Cataract Incidence in a Cohort of Mayak PA Workers following Chronic Occupational Radiation Exposure, *PloS one*, 11 (2016) e0164357.
- [28] W. Ruhm, G.E. Woloschak, R.E. Shore, T.V. Azizova, B. Grosche, O. Niwa, S. Akiba, T. Ono, K. Suzuki, T. Iwasaki, N. Ban, M. Kai, C.H. Clement, S. Bouffler, H. Toma, N. Hamada, Dose and dose-rate effects of ionizing radiation: a discussion in the light of radiological protection, *Radiation and environmental biophysics*, 54 (2015) 379-401.
- [29] F.A. Stewart, A.V. Akleyev, M. Hauer-Jensen, J.H. Hendry, N.J. Kleiman, T.J. Macvittie, B.M. Aleman, A.B. Edgar, K. Mabuchi, C.R. Muirhead, R.E. Shore, W.H. Wallace, ICRP publication 118: ICRP statement on tissue reactions and early and late effects of radiation in normal tissues and organs--threshold doses for tissue reactions in a radiation protection context, *Annals of the ICRP*, 41 (2012) 1-322.
- [30] S.P. Jackson, J. Bartek, The DNA-damage response in human biology and disease, *Nature*, 461 (2009) 1071-1078.
- [31] S.S. Khan, B.D. Singer, D.E. Vaughan, Molecular and physiological manifestations and measurement of aging in humans, *Aging cell*, 16 (2017) 624-633.
- [32] X.M. Caravia, D. Roiz-Valle, A. Moran-Alvarez, C. Lopez-Otin, Functional relevance of miRNAs in premature ageing, *Mechanisms of ageing and development*, 168 (2017) 10-19.
- [33] J. Campisi, J. Vijg, Does damage to DNA and other macromolecules play a role in aging? If so, how?, *The journals of gerontology. Series A, Biological sciences and medical sciences*, 64 (2009) 175-178.
- [34] E. Babusikova, J. Hatok, D. Dobrota, P. Kaplan, Age-related oxidative modifications of proteins and lipids in rat brain, *Neurochemical research*, 32 (2007) 1351-1356.
- [35] C. Lopez-Otin, L. Galluzzi, J.M.P. Freije, F. Madeo, G. Kroemer, Metabolic Control of Longevity, *Cell*, 166 (2016) 802-821.
- [36] D. Harman, Aging: a theory based on free radical and radiation chemistry, *Journal of gerontology*, 11 (1956) 298-300.
- [37] B.P. Yu, R. Yang, Critical evaluation of the free radical theory of aging. A proposal for the oxidative stress hypothesis, *Annals of the New York Academy of Sciences*, 786 (1996) 1-11.
- [38] T. Finkel, N.J. Holbrook, Oxidants, oxidative stress and the biology of ageing, *Nature*, 408 (2000) 239-247.
- [39] A. Golubev, A.D. Hanson, V.N. Gladyshev, Non-enzymatic molecular damage as a prototypic driver of aging, *The Journal of biological chemistry*, 292 (2017) 6029-6038.

- [40] D. Yin, K. Chen, The essential mechanisms of aging: Irreparable damage accumulation of biochemical side-reactions, *Experimental gerontology*, 40 (2005) 455-465.
- [41] T.J. Lyons, A.J. Jenkins, Glycation, oxidation, and lipoxidation in the development of the complications of diabetes: a carbonyl stress hypothesis, *Diabetes reviews* (Alexandria, Va.), 5 (1997) 365-391.
- [42] V.N. Gladyshev, The free radical theory of aging is dead. Long live the damage theory!, *Antioxidants & redox signaling*, 20 (2014) 727-731.
- [43] B.K. Kennedy, S.L. Berger, A. Brunet, J. Campisi, A.M. Cuervo, E.S. Epel, C. Franceschi, G.J. Lithgow, R.I. Morimoto, J.E. Pessin, T.A. Rando, A. Richardson, E.E. Schadt, T. Wyss-Coray, F. Sierra, Geroscience: linking aging to chronic disease, *Cell*, 159 (2014) 709-713.
- [44] C. Franceschi, P. Garagnani, C. Morsiani, M. Conte, A. Santoro, A. Grignolio, D. Monti, M. Capri, S. Salvioli, The Continuum of Aging and Age-Related Diseases: Common Mechanisms but Different Rates, *Frontiers in medicine*, 5 (2018) 61.
- [45] J. Cadet, J.R. Wagner, Oxidatively generated base damage to cellular DNA by hydroxyl radical and one-electron oxidants: similarities and differences, *Archives of biochemistry and biophysics*, 557 (2014) 47-54.
- [46] J.A. Reisz, N. Bansal, J. Qian, W. Zhao, C.M. Furdui, Effects of ionizing radiation on biological molecules--mechanisms of damage and emerging methods of detection, *Antioxidants & redox signaling*, 21 (2014) 260-292.
- [47] C.J. Lord, A. Ashworth, The DNA damage response and cancer therapy, *Nature*, 481 (2012) 287-294.
- [48] F. Faggioli, T. Wang, J. Vijg, C. Montagna, Chromosome-specific accumulation of aneuploidy in the aging mouse brain, *Human molecular genetics*, 21 (2012) 5246-5253.
- [49] L.A. Forsberg, C. Rasi, H.R. Razzaghian, G. Pakalapati, L. Waite, K.S. Thilbeault, A. Ronowicz, N.E. Wineinger, H.K. Tiwari, D. Boomsma, M.P. Westerman, J.R. Harris, R. Lyle, M. Essand, F. Eriksson, T.L. Assimes, C. Iribarren, E. Strachan, T.P. O'Hanlon, L.G. Rider, F.W. Miller, V. Giedraitis, L. Lannfelt, M. Ingelsson, A. Piotrowski, N.L. Pedersen, D. Absher, J.P. Dumanski, Age-related somatic structural changes in the nuclear genome of human blood cells, *American journal of human genetics*, 90 (2012) 217-228.
- [50] H. Li, J.R. Mitchell, P. Hasty, DNA double-strand breaks: a potential causative factor for mammalian aging?, *Mechanisms of ageing and development*, 129 (2008) 416-424.
- [51] E.G. Seviour, S.Y. Lin, The DNA damage response: Balancing the scale between cancer and ageing, *Aging* (Albany NY), 2 (2010) 900-907.
- [52] C.H. Bassing, F.W. Alt, The cellular response to general and programmed DNA double strand breaks, *DNA Repair* (Amst), 3 (2004) 781-796.

- [53] E. Riballo, M. Kuhne, N. Rief, A. Doherty, G.C. Smith, M.J. Recio, C. Reis, K. Dahm, A. Fricke, A. Krempler, A.R. Parker, S.P. Jackson, A. Gennery, P.A. Jeggo, M. Lobrich, A pathway of double-strand break rejoining dependent upon ATM, Artemis, and proteins locating to gamma-H2AX foci, *Mol Cell*, 16 (2004) 715-724.
- [54] T. Sperka, Z. Song, Y. Morita, K. Nalapareddy, L.M. Guachalla, A. Lechel, Y. Begus-Nahrman, M.D. Burkhalter, M. Mach, F. Schlaudraff, B. Liss, Z. Ju, M.R. Speicher, K.L. Rudolph, Puma and p21 represent cooperating checkpoints limiting self-renewal and chromosomal instability of somatic stem cells in response to telomere dysfunction, *Nat Cell Biol*, 14 (2011) 73-79.
- [55] M.S. Siddiqui, M. Francois, M.F. Fenech, W.R. Leifert, gammaH2AX responses in human buccal cells exposed to ionizing radiation, *Cytometry A*, 87 (2015) 296-308.
- [56] D. Brnzei, M. Foiani, Regulation of DNA repair throughout the cell cycle, *Nat Rev Mol Cell Biol*, 9 (2008) 297-308.
- [57] D.B. Lombard, K.F. Chua, R. Mostoslavsky, S. Franco, M. Gostissa, F.W. Alt, DNA repair, genome stability, and aging, *Cell*, 120 (2005) 497-512.
- [58] B. Schumacher, G.A. Garinis, J.H. Hoeijmakers, Age to survive: DNA damage and aging, *Trends in genetics : TIG*, 24 (2008) 77-85.
- [59] E.H. Blackburn, C.W. Greider, J.W. Szostak, Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging, *Nat Med*, 12 (2006) 1133-1138.
- [60] L. Kazak, A. Reyes, I.J. Holt, Minimizing the damage: repair pathways keep mitochondrial DNA intact, *Nat Rev Mol Cell Biol*, 13 (2012) 659-671.
- [61] S. Kuka, Z. Tatarkova, P. Kaplan, Oxidative damage to proteins and lipids during ageing, *Acta Martiniana*, 12 (2012) 5-11.
- [62] C.L. Klaips, G.G. Jayaraj, F.U. Hartl, Pathways of cellular proteostasis in aging and disease, *The Journal of cell biology*, 217 (2018) 51-63.
- [63] B.H. Toyama, M.W. Hetzer, Protein homeostasis: live long, won't prosper, *Nat Rev Mol Cell Biol*, 14 (2013) 55-61.
- [64] A. Ciechanover, Proteolysis: from the lysosome to ubiquitin and the proteasome, *Nat Rev Mol Cell Biol*, 6 (2005) 79-87.
- [65] M. Martinez-Vicente, G. Sovak, A.M. Cuervo, Protein degradation and aging, *Experimental gerontology*, 40 (2005) 622-633.
- [66] R. Pamplona, Membrane phospholipids, lipoxidative damage and molecular integrity: a causal role in aging and longevity, *Biochimica et biophysica acta*, 1777 (2008) 1249-1262.

- [67] B. Halliwell, J.M. Gutteridge, Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts, *Archives of biochemistry and biophysics*, 246 (1986) 501-514.
- [68] X. Han, H. Cheng, D.J. Mancuso, R.W. Gross, Caloric restriction results in phospholipid depletion, membrane remodeling, and triacylglycerol accumulation in murine myocardium, *Biochemistry*, 43 (2004) 15584-15594.
- [69] A.F. McDaid, P.K. Joshi, E. Porcu, A. Komljenovic, H. Li, V. Sorrentino, M. Litovchenko, R.P.J. Bevers, S. Rueger, A. Reymond, M. Bochud, B. Deplancke, R.W. Williams, M. Robinson-Rechavi, F. Paccaud, V. Rousson, J. Auwerx, J.F. Wilson, Z. Kutalik, Bayesian association scan reveals loci associated with human lifespan and linked biomarkers, *Nature communications*, 8 (2017) 15842.
- [70] P.R. Timmers, N. Mounier, K. Lall, K. Fischer, Z. Ning, X. Feng, A.D. Bretherick, D.W. Clark, M. Agbessi, H. Ahsan, I. Alves, A. Andiappan, P. Awadalla, A. Battle, M.J. Bonder, D. Boomsma, M. Christiansen, A. Claringbould, P. Deelen, J. van Dongen, T. Esko, M. Fave, L. Franke, T. Frayling, S.A. Gharib, G. Gibson, G. Hemani, R. Jansen, A. Kalnainen, S. Kasela, J. Kettunen, Y. Kim, H. Kirsten, P. Kovacs, K. Krohn, J. Kronberg-Guzman, V. Kukushkina, Z. Kutalik, M. Kahonen, B. Lee, T. Lehtimäki, M. Loeffler, U. Marigorta, A. Metspalu, J. van Meurs, L. Milani, M. Muller-Nurasyid, M. Nauck, M. Nivard, B. Penninx, M. Perola, N. Pervjakova, B. Pierce, J. Powell, H. Prokisch, B.M. Psaty, O. Raitakari, S. Ring, S. Ripatti, O. Rotzschke, S. Rueger, A. Saha, M. Scholz, K. Schramm, I. Seppala, M. Stumvoll, P. Sullivan, A. Teumer, J. Thiery, L. Tong, A. Tonjes, J. Verlouw, P.M. Visscher, U. Vosa, U. Volker, H. Yaghootkar, J. Yang, B. Zeng, F. Zhang, M. Agbessi, H. Ahsan, I. Alves, A. Andiappan, P. Awadalla, A. Battle, M.J. Bonder, D. Boomsma, M. Christiansen, A. Claringbould, P. Deelen, J. van Dongen, T. Esko, M. Fave, L. Franke, T. Frayling, S.A. Gharib, G. Gibson, G. Hemani, R. Jansen, A. Kalnainen, S. Kasela, J. Kettunen, Y. Kim, H. Kirsten, P. Kovacs, K. Krohn, J. Kronberg-Guzman, V. Kukushkina, Z. Kutalik, M. Kahonen, B. Lee, T. Lehtimäki, M. Loeffler, U. Marigorta, A. Metspalu, J. van Meurs, L. Milani, M. Muller-Nurasyid, M. Nauck, M. Nivard, B. Penninx, M. Perola, N. Pervjakova, B. Pierce, J. Powell, H. Prokisch, B.M. Psaty, O. Raitakari, S. Ring, S. Ripatti, O. Rotzschke, S. Rueger, A. Saha, M. Scholz, K. Schramm, I. Seppala, M. Stumvoll, P. Sullivan, A. Teumer, J. Thiery, L. Tong, A. Tonjes, J. Verlouw, P.M. Visscher, U. Vosa, U. Volker, H. Yaghootkar, J. Yang, B. Zeng, F. Zhang, X. Shen, T. Esko, Z. Kutalik, J.F. Wilson, P.K. Joshi, Genomics of 1 million parent lifespans implicates novel pathways and common diseases and distinguishes survival chances, *eLife*, 8 (2019).
- [71] T.O. Kilpeläinen, A.R. Bentley, R. Noordam, Y.J. Sung, K. Schwander, T.W. Winkler, H. Jakupovic, D.I. Chasman, A. Manning, I. Ntalla, H. Aschard, M.R. Brown, L. de Las Fuentes, N. Franceschini, X. Guo, D. Vojinovic, S. Aslibekyan, M.F. Feitosa, M. Kho, S.K. Musani, M. Richard, H. Wang, Z. Wang, T.M. Bartz, L.F. Bielak, A. Campbell, R. Dorajoo, V. Fisher, F.P. Hartwig, A. Horimoto, C. Li, K.K. Lohman, J. Marten, X. Sim, A.V. Smith, S.M. Tajuddin, M. Alver, M. Amini, M. Boissel, J.F. Chai, X. Chen, J. Divers, E. Evangelou, C. Gao, M. Graff, S.E. Harris, M. He, F.C. Hsu, A.U. Jackson, J.H. Zhao, A.T. Kraja, B. Kuhnle, F. Laguzzi, L.P. Lyytikäinen, I.M. Nolte, R. Rauramaa, M. Riaz, A. Robino, R. Rueedi, H.M. Stringham, F. Takeuchi, P.J. van der Most, T.V. Varga, N. Verweij, E.B. Ware, W. Wen, X. Li, L.R. Yanek, N. Amin, D.K. Arnett, E. Boerwinkle, M. Brumat, B. Cade, M. Canouil, Y.I. Chen, M.P. Concas, J. Connell, R. de Mutsert, H.J. de Silva, P.S. de Vries, A. Demirkan, J. Ding, C.B. Eaton, J.D. Faul, Y. Friedlander, K.P. Gabriel, M. Ghanbari, F. Giulianini, C.C. Gu, D. Gu, T.B. Harris, J. He, S.

Heikkinen, C.K. Heng, S.C. Hunt, M.A. Ikram, J.B. Jonas, W.P. Koh, P. Komulainen, J.E. Krieger, S.B. Kritchevsky, Z. Kutalik, J. Kuusisto, C.D. Langefeld, C. Langenberg, L.J. Launer, K. Leander, R.N. Lemaitre, C.E. Lewis, J. Liang, J. Liu, R. Magi, A. Manichaikul, T. Meitinger, A. Metspalu, Y. Milaneschi, K.L. Mohlke, T.H. Mosley, Jr., A.D. Murray, M.A. Nalls, E.K. Nang, C.P. Nelson, S. Nona, J.M. Norris, C.V. Nwuba, J. O'Connell, N.D. Palmer, G.J. Papanicolaou, R. Pazoki, N.L. Pedersen, A. Peters, P.A. Peyser, O. Polasek, D.J. Porteous, A. Poveda, O.T. Raitakari, S.S. Rich, N. Risch, J.G. Robinson, L.M. Rose, I. Rudan, P.J. Schreiner, R.A. Scott, S.S. Sidney, M. Sims, J.A. Smith, H. Snieder, T. Sofer, J.M. Starr, B. Sternfeld, K. Strauch, H. Tang, K.D. Taylor, M.Y. Tsai, J. Tuomilehto, A.G. Uitterlinden, M.Y. van der Ende, D. van Heemst, T. Voortman, M. Waldenberger, P. Wennberg, G. Wilson, Y.B. Xiang, J. Yao, C. Yu, J.M. Yuan, W. Zhao, A.B. Zonderman, D.M. Becker, M. Boehnke, D.W. Bowden, U. de Faire, I.J. Deary, P. Elliott, T. Esko, B.I. Freedman, P. Froguel, P. Gasparini, C. Gieger, N. Kato, M. Laakso, T.A. Lakka, T. Lehtimäki, P.K.E. Magnusson, A.J. Oldehinkel, B. Penninx, N.J. Samani, X.O. Shu, P. van der Harst, J.V. Van Vliet-Ostaptchouk, P. Vollenweider, L.E. Wagenknecht, Y.X. Wang, N.J. Wareham, D.R. Weir, T. Wu, W. Zheng, X. Zhu, M.K. Evans, P.W. Franks, V. Gudnason, C. Hayward, B.L. Horta, T.N. Kelly, Y. Liu, K.E. North, A.C. Pereira, P.M. Ridker, E.S. Tai, R.M. van Dam, E.R. Fox, S.L.R. Kardia, C.T. Liu, D.O. Mook-Kanamori, M.A. Province, S. Redline, C.M. van Duijn, J.I. Rotter, C.B. Kooperberg, W.J. Gauderman, B.M. Psaty, K. Rice, P.B. Munroe, M. Fornage, L.A. Cupples, C.N. Rotimi, A.C. Morrison, D.C. Rao, R.J.F. Loos, Multi-ancestry study of blood lipid levels identifies four loci interacting with physical activity, *Nature communications*, 10 (2019) 376.

[72] L.S. Merkens, C. Wassif, K. Healy, A.S. Pappu, A.E. DeBarber, J.A. Penfield, R.A. Lindsay, J.B. Roulet, F.D. Porter, R.D. Steiner, Smith-Lemli-Opitz syndrome and inborn errors of cholesterol synthesis: summary of the 2007 SLO/RSH Foundation scientific conference sponsored by the National Institutes of Health, *Genetics in medicine : official journal of the American College of Medical Genetics*, 11 (2009) 359-364.

[73] E.J. Sigler, J.C. Randolph, J.I. Calzada, M.W. Wilson, B.G. Haik, Current management of Coats disease, *Survey of ophthalmology*, 59 (2014) 30-46.

[74] B.L. Schafer, R.W. Bishop, V.J. Kratunis, S.S. Kalinowski, S.T. Mosley, K.M. Gibson, R.D. Tanaka, Molecular cloning of human mevalonate kinase and identification of a missense mutation in the genetic disease mevalonic aciduria, *The Journal of biological chemistry*, 267 (1992) 13229-13238.

[75] W. Chen, S. Kubota, Y. Nishimura, S. Nozaki, S. Yamashita, T. Nakagawa, K. Kameda-Takemura, M. Menju, Y. Matsuzawa, I. Bjorkhem, G. Eggertsen, Y. Seyama, Genetic analysis of a Japanese cerebrotendinous xanthomatosis family: identification of a novel mutation in the adrenodoxin binding region of the CYP 27 gene, *Biochimica et biophysica acta*, 1317 (1996) 119-126.

[76] G.E. Herman, Disorders of cholesterol biosynthesis: prototypic metabolic malformation syndromes, *Human molecular genetics*, 12 Spec No 1 (2003) R75-88.

[77] P.A. Krakowiak, C.A. Wassif, L. Kratz, D. Cozma, M. Kovarova, G. Harris, A. Grinberg, Y. Yang, A.G. Hunter, M. Tsokos, R.I. Kelley, F.D. Porter, Lathosterolosis: an inborn error of human and murine cholesterol synthesis due to lathosterol 5-desaturase deficiency, *Human molecular genetics*, 12 (2003) 1631-1641.

- [78] R. Anderson, S. Rust, J. Ashworth, J. Clayton-Smith, R.L. Taylor, P.T. Clayton, A.A.M. Morris, Lathosterolosis: A Relatively Mild Case with Cataracts and Learning Difficulties, *JIMD reports*, 44 (2019) 79-84.
- [79] F. Beby, O. Roche, C. Burillon, P. Denis, Coats' disease and bilateral cataract in a child with Turner syndrome: a case report, *Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie*, 243 (2005) 1291-1293.
- [80] E. Cotlier, P. Rice, Cataracts in the Smith-Lemli-Opitz syndrome, *American journal of ophthalmology*, 72 (1971) 955-959.
- [81] R. Buchert, H. Tawamie, C. Smith, S. Uebe, A.M. Innes, B. Al Hallak, A.B. Ekici, H. Sticht, B. Schwarze, R.E. Lamont, J.S. Parboosingh, F.P. Bernier, R. Abou Jamra, A peroxisomal disorder of severe intellectual disability, epilepsy, and cataracts due to fatty acyl-CoA reductase 1 deficiency, *American journal of human genetics*, 95 (2014) 602-610.
- [82] J.A. Thomas, R.J. Mallis, Aging and oxidation of reactive protein sulfhydryls, *Experimental gerontology*, 36 (2001) 1519-1526.
- [83] A. Cournil, T.B. Kirkwood, If you would live long, choose your parents well, *Trends in genetics : TIG*, 17 (2001) 233-235.
- [84] B.D. Mitchell, W.C. Hsueh, T.M. King, T.I. Pollin, J. Sorkin, R. Agarwala, A.A. Schaffer, A.R. Shuldiner, Heritability of life span in the Old Order Amish, *Am J Med Genet*, 102 (2001) 346-352.
- [85] C.J. Kenyon, The genetics of ageing, *Nature*, 464 (2010) 504-512.
- [86] P. Sebastiani, N. Solovieff, A.T. Dewan, K.M. Walsh, A. Puca, S.W. Hartley, E. Melista, S. Andersen, D.A. Dworkis, J.B. Wilk, R.H. Myers, M.H. Steinberg, M. Montano, C.T. Baldwin, J. Hoh, T.T. Perls, Genetic signatures of exceptional longevity in humans, *PloS one*, 7 (2012) e29848.
- [87] M.F. Fraga, E. Ballestar, M.F. Paz, S. Ropero, F. Setien, M.L. Ballestar, D. Heine-Suner, J.C. Cigudosa, M. Urioste, J. Benitez, M. Boix-Chornet, A. Sanchez-Aguilera, C. Ling, E. Carlsson, P. Poulsen, A. Vaag, Z. Stephan, T.D. Spector, Y.Z. Wu, C. Plass, M. Esteller, Epigenetic differences arise during the lifetime of monozygotic twins, *Proc Natl Acad Sci U S A*, 102 (2005) 10604-10609.
- [88] R.P. Talens, K. Christensen, H. Putter, G. Willemsen, L. Christiansen, D. Kremer, H.E. Suchiman, P.E. Slagboom, D.I. Boomsma, B.T. Heijmans, Epigenetic variation during the adult lifespan: cross-sectional and longitudinal data on monozygotic twin pairs, *Aging cell*, 11 (2012) 694-703.
- [89] J. van Dongen, E.A. Ehli, R.C. Sliker, M. Bartels, Z.M. Weber, G.E. Davies, P.E. Slagboom, B.T. Heijmans, D.I. Boomsma, Epigenetic variation in monozygotic twins: a genome-wide analysis of DNA methylation in buccal cells, *Genes (Basel)*, 5 (2014) 347-365.

- [90] W.P. Vermeij, M.E. Dolle, E. Reiling, D. Jaarsma, C. Payan-Gomez, C.R. Bombardieri, H. Wu, A.J. Roks, S.M. Botter, B.C. van der Eerden, S.A. Youssef, R.V. Kuiper, B. Nagarajah, C.T. van Oostrom, R.M. Brandt, S. Barnhoorn, S. Imholz, J.L. Pennings, A. de Bruin, A. Gyenis, J. Pothof, J. Vijg, H. van Steeg, J.H. Hoeijmakers, Restricted diet delays accelerated ageing and genomic stress in DNA-repair-deficient mice, *Nature*, 537 (2016) 427-431.
- [91] A.K. Hedman, M. Zilmer, J. Sundstrom, L. Lind, E. Ingelsson, DNA methylation patterns associated with oxidative stress in an ageing population, *BMC Med Genomics*, 9 (2016) 72.
- [92] A. Busanello, D. Santucci, S. Bonini, A. Micera, Review: Environmental impact on ocular surface disorders: Possible epigenetic mechanism modulation and potential biomarkers, *Ocul Surf*, 15 (2017) 680-687.
- [93] J. Kochmanski, L. Montrose, J.M. Goodrich, D.C. Dolinoy, Environmental Deflection: The Impact of Toxicant Exposures on the Aging Epigenome, *Toxicol Sci*, 156 (2017) 325-335.
- [94] P.G. Shiels, D. McGuinness, M. Eriksson, J.P. Kooman, P. Stenvinkel, The role of epigenetics in renal ageing, *Nat Rev Nephrol*, 13 (2017) 471-482.
- [95] M.C. Haigis, B.A. Yankner, The aging stress response, *Mol Cell*, 40 (2010) 333-344.
- [96] L.C.D. Pomatto, K.J.A. Davies, The role of declining adaptive homeostasis in ageing, *The Journal of physiology*, 595 (2017) 7275-7309.
- [97] J.F. Fries, The compression of morbidity. 1983, *Milbank Q*, 83 (2005) 801-823.
- [98] N.M. Peel, R.J. McClure, H.P. Bartlett, Behavioral determinants of healthy aging, *Am J Prev Med*, 28 (2005) 298-304.
- [99] S. Song, A. Landsbury, R. Dahm, Y. Liu, Q. Zhang, R.A. Quinlan, Functions of the intermediate filament cytoskeleton in the eye lens, *J Clin Invest*, 119 (2009) 1837-1848.
- [100] P.J. Donaldson, A.C. Grey, B. Maceo Heilman, J.C. Lim, E. Vaghefi, The physiological optics of the lens, *Progress in retinal and eye research*, 56 (2017) e1-e24.
- [101] R.C. Augusteyn, On the growth and internal structure of the human lens, *Experimental eye research*, 90 (2010) 643-654.
- [102] S. Bassnett, Y. Shi, G.F. Vrensen, Biological glass: structural determinants of eye lens transparency, *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 366 (2011) 1250-1264.
- [103] R.T. Mathias, J. Kistler, P. Donaldson, The lens circulation, *The Journal of membrane biology*, 216 (2007) 1-16.
- [104] R.T. Mathias, T.W. White, X. Gong, Lens gap junctions in growth, differentiation, and homeostasis, *Physiological reviews*, 90 (2010) 179-206.

- [105] L.A. Brennan, R.S. McGreal, M. Kantorow, Oxidative stress defense and repair systems of the ocular lens, *Frontiers in bioscience (Elite edition)*, 4 (2012) 141-155.
- [106] B. Wang, G. Hom, S. Zhou, M. Guo, B. Li, J. Yang, V.M. Monnier, X. Fan, The oxidized thiol proteome in aging and cataractous mouse and human lens revealed by ICAT labeling, *Aging cell*, 16 (2017) 244-261.
- [107] X. Fan, V.M. Monnier, J. Whitson, Lens glutathione homeostasis: Discrepancies and gaps in knowledge standing in the way of novel therapeutic approaches, *Experimental eye research*, 156 (2017) 103-111.
- [108] N. Pescosolido, A. Barbato, R. Giannotti, C. Komaiha, F. Lenarduzzi, Age-related changes in the kinetics of human lenses: prevention of the cataract, *Int J Ophthalmol-Chi*, 9 (2016) 1506-1517.
- [109] D. Borchman, R. Stimmelmayer, J.C. George, Whales, lifespan, phospholipids, and cataracts, *Journal of lipid research*, 58 (2017) 2289-2298.
- [110] W.K. Subczynski, J. Widomska, L. Mainali, Factors Determining the Oxygen Permeability of Biological Membranes: Oxygen Transport Across Eye Lens Fiber-Cell Plasma Membranes, *Advances in experimental medicine and biology*, 977 (2017) 27-34.
- [111] N. Hamada, Y. Fujimichi, T. Iwasaki, N. Fujii, M. Furuhashi, E. Kubo, T. Minamino, T. Nomura, H. Sato, Emerging issues in radiogenic cataracts and cardiovascular disease, *Journal of radiation research*, 55 (2014) 831-846.
- [112] M.H. Sweeney, R.J. Truscott, An impediment to glutathione diffusion in older normal human lenses: a possible precondition for nuclear cataract, *Experimental eye research*, 67 (1998) 587-595.
- [113] D.A. Goodenough, The crystalline lens. A system networked by gap junctional intercellular communication, *Seminars in cell biology*, 3 (1992) 49-58.
- [114] L. Kaestner, G. Minetti, The potential of erythrocytes as cellular aging models, *Cell death and differentiation*, 24 (2017) 1475-1477.
- [115] T. Mathieson, H. Franken, J. Kosinski, N. Kurzawa, N. Zinn, G. Sweetman, D. Poeckel, V.S. Ratnu, M. Schramm, I. Becher, M. Steidel, K.M. Noh, G. Bergamini, M. Beck, M. Bantscheff, M.M. Savitski, Systematic analysis of protein turnover in primary cells, *Nature communications*, 9 (2018) 689.
- [116] J. Nielsen, R.B. Hedeholm, J. Heinemeier, P.G. Bushnell, J.S. Christiansen, J. Olsen, C.B. Ramsey, R.W. Brill, M. Simon, K.F. Steffensen, J.F. Steffensen, Eye lens radiocarbon reveals centuries of longevity in the Greenland shark (*Somniosus microcephalus*), *Science (New York, N.Y.)*, 353 (2016) 702-704.
- [117] S.J. McGinty, R.J. Truscott, Presbyopia: the first stage of nuclear cataract?, *Ophthalmic research*, 38 (2006) 137-148.

- [118] R. Michael, A.J. Bron, The ageing lens and cataract: a model of normal and pathological ageing, *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 366 (2011) 1278-1292.
- [119] M.C. Leske, S.Y. Wu, B. Nemesure, L. Yang, A. Hennis, Nine-year incidence of lens opacities in the Barbados Eye Studies, *Ophthalmology*, 111 (2004) 483-490.
- [120] H. Sasaki, F. Jonasson, Y.B. Shui, M. Kojima, M. Ono, N. Katoh, H.M. Cheng, N. Takahashi, K. Sasaki, High prevalence of nuclear cataract in the population of tropical and subtropical areas, *Developments in ophthalmology*, 35 (2002) 60-69.
- [121] T. Neumayer, N. Hirschschall, M. Georgopoulos, O. Findl, Natural course of posterior subcapsular cataract over a short time period, *Current eye research*, 42 (2017) 1604-1607.
- [122] V.N. Gladyshev, Aging: progressive decline in fitness due to the rising deleteriome adjusted by genetic, environmental, and stochastic processes, *Aging cell*, 15 (2016) 594-602.
- [123] V.D. Longo, J. Mitteldorf, V.P. Skulachev, Programmed and altruistic ageing, *Nature reviews. Genetics*, 6 (2005) 866-872.
- [124] T.B. Kirkwood, Evolution of ageing, *Nature*, 270 (1977) 301-304.
- [125] M.V. Blagosklonny, Aging: ROS or TOR, *Cell cycle (Georgetown, Tex.)*, 7 (2008) 3344-3354.
- [126] A. Shiels, J.F. Hejtmancik, Mutations and mechanisms in congenital and age-related cataracts, *Experimental eye research*, 156 (2017) 95-102.
- [127] L.J. Dawes, G. Duncan, I.M. Wormstone, Age-related differences in signaling efficiency of human lens cells underpin differential wound healing response rates following cataract surgery, *Investigative ophthalmology & visual science*, 54 (2013) 333-342.
- [128] B.D. Hood, B. Garner, R.J. Truscott, Human lens coloration and aging. Evidence for crystallin modification by the major ultraviolet filter, 3-hydroxy-kynurenine O-beta-D-glucoside, *The Journal of biological chemistry*, 274 (1999) 32547-32550.
- [129] H. Girao, M.C. Mota, J. Ramalho, P. Pereira, Cholesterol oxides accumulate in human cataracts, *Experimental eye research*, 66 (1998) 645-652.
- [130] I. Guggenmoos-Holzmam, B. Engel, V. Henke, G.O. Naumann, Cell density of human lens epithelium in women higher than in men, *Investigative ophthalmology & visual science*, 30 (1989) 330-332.
- [131] J.J. Wu, W. Wu, F.M. Tholozan, C.D. Saunter, J.M. Girkin, R.A. Quinlan, A dimensionless ordered pull-through model of the mammalian lens epithelium evidences scaling across species and explains the age-dependent changes in cell density in the

human lens, *Journal of the Royal Society, Interface / the Royal Society*, 12 (2015) 20150391.

[132] W. Pendergrass, G. Zitnik, R. Tsai, N. Wolf, X-ray induced cataract is preceded by LEC loss, and coincident with accumulation of cortical DNA, and ROS; similarities with age-related cataracts, *Molecular vision*, 16 (2010) 1496-1513.

[133] J.R. Kuszak, A re-examination of primate lens epithelial cell size, density and structure as a function of development, growth and age., *Nova Acta Leopoldina*, 57 (1997) 45-66.

[134] Y.B. Shui, D.C. Beebe, Age-dependent control of lens growth by hypoxia, *Investigative ophthalmology & visual science*, 49 (2008) 1023-1029.

[135] L. Von Sallmann, Experimental studies on early lens changes after roentgen irradiation. III. Effect of x-radiation on mitotic activity and nuclear fragmentation of lens epithelium in normal and cysteine-treated rabbits, *A.M.A. archives of ophthalmology*, 47 (1952) 305-320.

[136] R.C. Augusteyn, Growth of the eye lens: II. Allometric studies, *Molecular vision*, 20 (2014) 427-440.

[137] F. Li, Y. Wang, G. Zhang, J. Zhou, L. Yang, H. Guan, Expression and methylation of DNA repair genes in lens epithelium cells of age-related cataract, *Mutation research*, 766-767 (2014) 31-36.

[138] B. Xu, L. Kang, G. Zhang, J. Wu, R. Zhu, M. Yang, H. Guan, The changes of 8-OHdG, hOGG1, APE1 and Pol beta in lenses of patients with age-related cataract, *Current eye research*, 40 (2015) 378-385.

[139] Y. Wang, F. Li, G. Zhang, L. Kang, H. Guan, Ultraviolet-B induces ERCC6 repression in lens epithelium cells of age-related nuclear cataract through coordinated DNA hypermethylation and histone deacetylation, *Clinical epigenetics*, 8 (2016) 62.

[140] X.Q. Huang, J. Wang, J.P. Liu, H. Feng, W.B. Liu, Q. Yan, Y. Liu, S.M. Sun, M. Deng, L. Gong, Y. Liu, D.W. Li, hTERT extends proliferative lifespan and prevents oxidative stress-induced apoptosis in human lens epithelial cells, *Investigative ophthalmology & visual science*, 46 (2005) 2503-2513.

[141] M.A. Babizhayev, K.S. Vishnyakova, Y.E. Yegorov, Telomere-dependent senescent phenotype of lens epithelial cells as a biological marker of aging and cataractogenesis: the role of oxidative stress intensity and specific mechanism of phospholipid hydroperoxide toxicity in lens and aqueous, *Fundamental & clinical pharmacology*, 25 (2011) 139-162.

[142] M.A. Babizhayev, Y.E. Yegorov, Telomere Attrition in Human Lens Epithelial Cells Associated with Oxidative Stress Provide a New Therapeutic Target for the Treatment, Dissolving and Prevention of Cataract with N-Acetylcarnosine Lubricant Eye Drops. Kinetic, Pharmacological and Activity-Dependent Separation of Therapeutic Targeting: Transcorneal Penetration and Delivery of L-Carnosine in the Aqueous Humor and Hormone-Like Hypothalamic Antiaging Effects of the Instilled Ophthalmic Drug Through

a Safe Eye Medication Technique, Recent patents on drug delivery & formulation, 10 (2016) 82-129.

[143] S. Ottonello, C. Foroni, A. Carta, S. Petrucco, G. Maraini, Oxidative stress and age-related cataract, *Ophthalmologica. Journal international d'ophtalmologie. International journal of ophthalmology. Zeitschrift fur Augenheilkunde*, 214 (2000) 78-85.

[144] T.J. Kinsella, Understanding DNA damage response and DNA repair pathways: applications to more targeted cancer therapeutics, *Seminars in oncology*, 36 (2009) S42-51.

[145] S.K. West, C.T. Valmadrid, Epidemiology of risk factors for age-related cataract, *Survey of ophthalmology*, 39 (1995) 323-334.

[146] P.J. Foster, T.Y. Wong, D. Machin, G.J. Johnson, S.K. Seah, Risk factors for nuclear, cortical and posterior subcapsular cataracts in the Chinese population of Singapore: the Tanjong Pagar Survey, *The British journal of ophthalmology*, 87 (2003) 1112-1120.

[147] P.T. Williams, Walking and running are associated with similar reductions in cataract risk, *Medicine and science in sports and exercise*, 45 (2013) 1089-1096.

[148] L. Robman, H. Taylor, External factors in the development of cataract, *Eye (London, England)*, 19 (2005) 1074-1082.

[149] S. Lerman, Evaluation of risk factors in human cataractogenesis, *Developments in ophthalmology*, 21 (1991) 120-128.

[150] K.A. Weikel, C. Garber, A. Baburins, A. Taylor, Nutritional modulation of cataract, *Nutrition reviews*, 72 (2014) 30-47.

[151] K. Kato, H. Shinohara, N. Kurobe, S. Goto, Y. Inaguma, K. Ohshima, Immunoreactive alpha A crystallin in rat non-lenticular tissues detected with a sensitive immunoassay method, *Biochimica et biophysica acta*, 1080 (1991) 173-180.

[152] K. Kato, H. Shinohara, N. Kurobe, Y. Inaguma, K. Shimizu, K. Ohshima, Tissue distribution and developmental profiles of immunoreactive aB crystallin in the rat non-lenticular tissues determined with a sensitive immunoassay system, *Biochimica et biophysica acta*, 1074 (1991) 201-208.

[153] A.R. Clark, N.H. Lubsen, C. Slingsby, sHSP in the eye lens: crystallin mutations, cataract and proteostasis, *The international journal of biochemistry & cell biology*, 44 (2012) 1687-1697.

[154] P. Pereira, F. Shang, M. Hobbs, H. Girao, A. Taylor, Lens fibers have a fully functional ubiquitin-proteasome pathway, *Experimental eye research*, 76 (2003) 623-631.

[155] N. Lieska, K. Krotzer, H.Y. Yang, A reassessment of protein synthesis by lens nuclear fiber cells, *Experimental eye research*, 54 (1992) 807-811.

- [156] J. Qian, R.M. Lavker, H. Tseng, Mapping ribosomal RNA transcription activity in the mouse eye, *Developmental dynamics : an official publication of the American Association of Anatomists*, 235 (2006) 1984-1993.
- [157] F. Imai, A. Yoshizawa, N. Fujimori-Tonou, K. Kawakami, I. Masai, The ubiquitin proteasome system is required for cell proliferation of the lens epithelium and for differentiation of lens fiber cells in zebrafish, *Development (Cambridge, England)*, 137 (2010) 3257-3268.
- [158] H. Morishita, S. Eguchi, H. Kimura, J. Sasaki, Y. Sakamaki, M.L. Robinson, T. Sasaki, N. Mizushima, Deletion of autophagy-related 5 (Atg5) and Pik3c3 genes in the lens causes cataract independent of programmed organelle degradation, *The Journal of biological chemistry*, 288 (2013) 11436-11447.
- [159] K. Liu, L. Lyu, D. Chin, J. Gao, X. Sun, F. Shang, A. Caceres, M.L. Chang, S. Rowan, J. Peng, R. Mathias, H. Kasahara, S. Jiang, A. Taylor, Altered ubiquitin causes perturbed calcium homeostasis, hyperactivation of calpain, dysregulated differentiation, and cataract, *Proc Natl Acad Sci U S A*, 112 (2015) 1071-1076.
- [160] H. Morishita, N. Mizushima, Autophagy in the lens, *Experimental eye research*, 144 (2016) 22-28.
- [161] Y.A. Lyon, G.M. Sabbah, R.R. Julian, Identification of Sequence Similarities among Isomerization Hotspots in Crystallin Proteins, *Journal of proteome research*, 16 (2017) 1797-1805.
- [162] K.J. Lampi, P.A. Wilmarth, M.R. Murray, L.L. David, Lens beta-crystallins: the role of deamidation and related modifications in aging and cataract, *Progress in biophysics and molecular biology*, 115 (2014) 21-31.
- [163] A. Taylor, K.J. Davies, Protein oxidation and loss of protease activity may lead to cataract formation in the aged lens, *Free radical biology & medicine*, 3 (1987) 371-377.
- [164] E.L. Siew, D. Opalecky, F.A. Bettelheim, Light scattering of normal human lens. II. Age dependence of the light scattering parameters, *Experimental eye research*, 33 (1981) 603-614.
- [165] F. Boscia, I. Grattagliano, G. Vendemiale, T. Micelli-Ferrari, E. Altomare, Protein oxidation and lens opacity in humans, *Investigative ophthalmology & visual science*, 41 (2000) 2461-2465.
- [166] F.J. Giblin, Glutathione: a vital lens antioxidant, *Journal of ocular pharmacology and therapeutics : the official journal of the Association for Ocular Pharmacology and Therapeutics*, 16 (2000) 121-135.
- [167] L. Huang, V. Grami, Y. Marrero, D. Tang, M.C. Yappert, V. Rasi, D. Borchman, Human lens phospholipid changes with age and cataract, *Investigative ophthalmology & visual science*, 46 (2005) 1682-1689.

- [168] D. Borchman, N.A. Delamere, L.A. McCauley, C.A. Paterson, Studies on the distribution of cholesterol, phospholipid, and protein in the human and bovine lens, *Lens and eye toxicity research*, 6 (1989) 703-724.
- [169] A.C. de Vries, M.A. Vermeer, A.L. Hendriks, H. Bloemendal, L.H. Cohen, Biosynthetic capacity of the human lens upon aging, *Experimental eye research*, 53 (1991) 519-524.
- [170] E.M. Oborina, M.C. Yappert, Effect of sphingomyelin versus dipalmitoylphosphatidylcholine on the extent of lipid oxidation, *Chemistry and physics of lipids*, 123 (2003) 223-232.
- [171] D. Borchman, O.P. Lamba, S. Salmassi, M. Lou, M.C. Yappert, The dual effect of oxidation on lipid bilayer structure, *Lipids*, 27 (1992) 261-265.
- [172] P.C. Noordam, A. Killian, R.F. Oude Elferink, J. De Gier, Comparative study on the properties of saturated phosphatidylethanolamine and phosphatidylcholine bilayers: barrier characteristics and susceptibility to phospholipase A2 degradation, *Chemistry and physics of lipids*, 31 (1982) 191-204.
- [173] D. Borchman, M.C. Yappert, M. Afzal, Lens lipids and maximum lifespan, *Experimental eye research*, 79 (2004) 761-768.
- [174] D. Tang, D. Borchman, A.K. Schwarz, M.C. Yappert, G.F. Vrensen, J. van Marle, D.B. DuPre, Light scattering of human lens vesicles in vitro, *Experimental eye research*, 76 (2003) 605-612.
- [175] E. Plesnar, R. Szczelina, W.K. Subczynski, M. Pasenkiewicz-Gierula, Is the cholesterol bilayer domain a barrier to oxygen transport into the eye lens?, *Biochimica et biophysica acta*, 1860 (2018) 434-441.
- [176] D. Borchman, D. Tang, Binding capacity of alpha-crystallin to bovine lens lipids, *Experimental eye research*, 63 (1996) 407-410.
- [177] S.L. Reichow, T. Gonen, Lipid-protein interactions probed by electron crystallography, *Current opinion in structural biology*, 19 (2009) 560-565.
- [178] J. Tong, J.T. Canty, M.M. Briggs, T.J. McIntosh, The water permeability of lens aquaporin-0 depends on its lipid bilayer environment, *Experimental eye research*, 113 (2013) 32-40.
- [179] D. Tang, D. Borchman, M.C. Yappert, R.J. Cenedella, Influence of cholesterol on the interaction of alpha-crystallin with phospholipids, *Experimental eye research*, 66 (1998) 559-567.
- [180] L.N. Makley, K.A. McMenimen, B.T. DeVree, J.W. Goldman, B.N. McGlasson, P. Rajagopal, B.M. Dunyak, T.J. McQuade, A.D. Thompson, R. Sunahara, R.E. Klevit, U.P. Andley, J.E. Gestwicki, Pharmacological chaperone for alpha-crystallin partially restores transparency in cataract models, *Science (New York, N.Y.)*, 350 (2015) 674-677.

- [181] L. Zhao, X.J. Chen, J. Zhu, Y.B. Xi, X. Yang, L.D. Hu, H. Ouyang, S.H. Patel, X. Jin, D. Lin, F. Wu, K. Flagg, H. Cai, G. Li, G. Cao, Y. Lin, D. Chen, C. Wen, C. Chung, Y. Wang, A. Qiu, E. Yeh, W. Wang, X. Hu, S. Grob, R. Abagyan, Z. Su, H.C. Tjondro, X.J. Zhao, H. Luo, R. Hou, J.J. Perry, W. Gao, I. Kozak, D. Granet, Y. Li, X. Sun, J. Wang, L. Zhang, Y. Liu, Y.B. Yan, K. Zhang, Lanosterol reverses protein aggregation in cataracts, *Nature*, 523 (2015) 607-611.
- [182] H. Girao, P. Pereira, J. Ramalho, R. Quinlan, A. Prescott, Cholesterol oxides mediated changes in cytoskeletal organisation involves Rho GTPases, *Experimental cell research*, 291 (2003) 502-513.
- [183] H. Girao, S. Catarino, P. Pereira, 7-Ketocholesterol modulates intercellular communication through gap-junction in bovine lens epithelial cells, *Cell communication and signaling : CCS*, 2 (2004) 2.
- [184] A.G. Abraham, N.G. Condon, E. West Gower, The new epidemiology of cataract, *Ophthalmology clinics of North America*, 19 (2006) 415-425.
- [185] C. Gilbert, H. Awan, Blindness in children, *BMJ (Clinical research ed.)*, 327 (2003) 760-761.
- [186] C. Gilbert, J. Rahi, M. Eckstein, A. Foster, Hereditary disease as a cause of childhood blindness: regional variation. Results of blind school studies undertaken in countries of Latin America, Asia and Africa, *Ophthalmic genetics*, 16 (1995) 1-10.
- [187] D.E. Kreuger, R.C. Milton, L.R. Maunder, The Framingham eye study: introduction to the monograph, *Survey of ophthalmology*, 24 (1980) 614-620.
- [188] B.E. Klein, R. Klein, K.E. Lee, Incidence of age-related cataract: the Beaver Dam Eye Study, *Archives of ophthalmology (Chicago, Ill. : 1960)*, 116 (1998) 219-225.
- [189] C.A. McCarty, H.R. Taylor, The genetics of cataract, *Investigative ophthalmology & visual science*, 42 (2001) 1677-1678.
- [190] Y. Okano, M. Asada, A. Fujimoto, A. Ohtake, K. Murayama, K.J. Hsiao, K. Choeh, Y. Yang, Q. Cao, J.K. Reichardt, S. Niihira, T. Imamura, T. Yamano, A genetic factor for age-related cataract: identification and characterization of a novel galactokinase variant, "Osaka," in Asians, *American journal of human genetics*, 68 (2001) 1036-1042.
- [191] B.E. Lindblad, N. Hakansson, A. Wolk, Smoking cessation and the risk of cataract: a prospective cohort study of cataract extraction among men, *JAMA ophthalmology*, 132 (2014) 253-257.
- [192] Y. Gong, K. Feng, N. Yan, Y. Xu, C.W. Pan, Different amounts of alcohol consumption and cataract: a meta-analysis, *Optometry and vision science : official publication of the American Academy of Optometry*, 92 (2015) 471-479.
- [193] R.E. Neale, J.L. Purdie, L.W. Hirst, A.C. Green, Sun exposure as a risk factor for nuclear cataract, *Epidemiology (Cambridge, Mass.)*, 14 (2003) 707-712.

- [194] W.G. Hodge, J.P. Whitche, W. Satariano, Risk factors for age-related cataracts, *Epidemiologic reviews*, 17 (1995) 336-346.
- [195] R.F. Liao, M.J. Ye, C.Y. Liu, D.Q. Ye, An Updated Meta-Analysis: Risk Conferred by Glutathione S-Transferases (GSTM1 and GSTT1) Polymorphisms to Age-Related Cataract, *Journal of ophthalmology*, 2015 (2015) 103950.
- [196] J. Jiang, J. Zhou, Y. Yao, R. Zhu, C. Liang, S. Jiang, M. Yang, Y. Lu, Q. Xing, H. Guan, Copy number variations of DNA repair genes and the age-related cataract: Jiangsu Eye Study, *Investigative ophthalmology & visual science*, 54 (2013) 932-938.
- [197] A. Shiels, T.M. Bennett, H.L. Knopf, G. Maraini, A. Li, X. Jiao, J.F. Hejtmancik, The EPHA2 gene is associated with cataracts linked to chromosome 1p, *Molecular vision*, 14 (2008) 2042-2055.
- [198] S. Jiang, N. Hu, J. Zhou, J. Zhang, R. Gao, J. Hu, H. Guan, Polymorphisms of the WRN gene and DNA damage of peripheral lymphocytes in age-related cataract in a Han Chinese population, *Age (Dordrecht, Netherlands)*, 35 (2013) 2435-2444.
- [199] G. Padma, M. Mamata, K.R. Reddy, T. Padma, Polymorphisms in two DNA repair genes (XPD and XRCC1)--association with age related cataracts, *Molecular vision*, 17 (2011) 127-133.
- [200] A. Asai, M. Matsutani, T. Kohno, O. Nakamura, H. Tanaka, T. Fujimaki, N. Funada, T. Matsuda, K. Nagata, K. Takakura, Subacute brain atrophy after radiation therapy for malignant brain tumor, *Cancer*, 63 (1989) 1962-1974.
- [201] S.C. Darby, P. McGale, C.W. Taylor, R. Peto, Long-term mortality from heart disease and lung cancer after radiotherapy for early breast cancer: prospective cohort study of about 300,000 women in US SEER cancer registries, *Lancet Oncol*, 6 (2005) 557-565.
- [202] X.R. Lowe, S. Bhattacharya, F. Marchetti, A.J. Wyrobek, Early brain response to low-dose radiation exposure involves molecular networks and pathways associated with cognitive functions, advanced aging and Alzheimer's disease, *Radiation research*, 171 (2009) 53-65.
- [203] Y. Shimizu, K. Kodama, N. Nishi, F. Kasagi, A. Suyama, M. Soda, E.J. Grant, H. Sugiyama, R. Sakata, H. Moriwaki, M. Hayashi, M. Konda, R.E. Shore, Radiation exposure and circulatory disease risk: Hiroshima and Nagasaki atomic bomb survivor data, 1950-2003, *BMJ (Clinical research ed.)*, 340 (2010) b5349.
- [204] O. Azimzadeh, W. Sievert, H. Sarioglu, J. Merl-Pham, R. Yentrapalli, M.V. Bakshi, D. Janik, M. Ueffing, M.J. Atkinson, G. Multhoff, S. Tapio, Integrative proteomics and targeted transcriptomics analyses in cardiac endothelial cells unravel mechanisms of long-term radiation-induced vascular dysfunction, *Journal of proteome research*, 14 (2015) 1203-1219.
- [205] O.N. Le, F. Rodier, F. Fontaine, J.P. Coppe, J. Campisi, J. DeGregori, C. Laverdiere, V. Kokta, E. Haddad, C.M. Beausejour, Ionizing radiation-induced long-term expression of

senescence markers in mice is independent of p53 and immune status, *Aging cell*, 9 (2010) 398-409.

[206] H. Chaluppecky, Ueber die wirkung der Roentgenstrahlen, *Centralblatt fuer praktische Augenheilkunde*, 21 (1897) 386-401.

[207] W. Rohrschneider, Experimentelle Untersuchungen über die Veränderungen normaler Augengewebe nach Röntgenbestrahlung. III. Veränderungen der Linse der Netzhaut und des Sehnerven nach Röntgenbestrahlung., *Albrecht von Graefes Arch Klin Exp Ophthalmol* 122 (1929) 282-290.

[208] D.G. Cogan, D.D. Donaldson, A.B. Reese, Clinical and pathological characteristics of radiation cataract, *A.M.A. archives of ophthalmology*, 47 (1952) 55-70.

[209] D.G. Cogan, S.F. Martin, S.J. Kimura, Atom bomb cataracts, *Science (New York, N.Y.)*, 110 (1949) 654.

[210] K. Choshi, I. Takaku, H. Mishima, T. Takase, S. Neriishi, S.C. Finch, M. Otake, Ophthalmologic changes related to radiation exposure and age in adult health study sample, Hiroshima and Nagasaki, *Radiation research*, 96 (1983) 560-579.

[211] R.L. Black, R.B. Oglesby, L. Von Sallmann, J.J. Bunim, Posterior subcapsular cataracts induced by corticosteroids in patients with rheumatoid arthritis, *Jama*, 174 (1960) 166-171.

[212] E.R. James, The etiology of steroid cataract, *Journal of ocular pharmacology and therapeutics : the official journal of the Association for Ocular Pharmacology and Therapeutics*, 23 (2007) 403-420.

[213] C.J. Brown, F. Akaichi, Vitamin D deficiency and posterior subcapsular cataract, *Clinical ophthalmology (Auckland, N.Z.)*, 9 (2015) 1093-1098.

[214] P. Sharma, A.R. Vasavada, Acute transient bilateral diabetic posterior subcapsular cataracts(1), *Journal of cataract and refractive surgery*, 27 (2001) 789-794.

[215] G. Adrien Shun-Shin, N.P. Brown, A.J. Bron, J.M. Sparrow, Dynamic nature of posterior subcapsular cataract, *The British journal of ophthalmology*, 73 (1989) 522-527.

[216] J.A. Jones, M. McCarten, K. Manuel, B. Djojonegoro, J. Murray, A. Feiveson, M. Wear, Cataract formation mechanisms and risk in aviation and space crews, *Aviation, space, and environmental medicine*, 78 (2007) A56-66.

[217] L.T. Chylack, Jr., L.E. Peterson, A.H. Feiveson, M.L. Wear, F.K. Manuel, W.H. Tung, D.S. Hardy, L.J. Marak, F.A. Cucinotta, NASA study of cataract in astronauts (NASCA). Report 1: Cross-sectional study of the relationship of exposure to space radiation and risk of lens opacity, *Radiation research*, 172 (2009) 10-20.

[218] L.T. Chylack, Jr., A.H. Feiveson, L.E. Peterson, W.H. Tung, M.L. Wear, L.J. Marak, D.S. Hardy, L.J. Chappell, F.A. Cucinotta, NASCA report 2: Longitudinal study of relationship of

exposure to space radiation and risk of lens opacity, *Radiation research*, 178 (2012) 25-32.

[219] J. Domienik, S. Gryglak, J. Jurewicz, Characteristics of interventional cardiologists and their work practices for the study on radiation-induced lens opacities based on the methodology developed by ELDO-preliminary results, *Journal of radiation research*, 57 (2016) 431-437.

[220] S.L. Simon, D.L. Preston, M.S. Linet, J.S. Miller, A.J. Sigurdson, B.H. Alexander, D. Kwon, R.C. Yoder, P. Bhatti, M.P. Little, P. Rajaraman, D. Melo, V. Drozdovitch, R.M. Weinstock, M.M. Doody, Radiation organ doses received in a nationwide cohort of U.S. radiologic technologists: methods and findings, *Radiation research*, 182 (2014) 507-528.

[221] M.P. Little, C.M. Kitahara, E.K. Cahoon, M.O. Bernier, R. Velazquez-Kronen, M.M. Doody, D. Borrego, J.S. Miller, B.H. Alexander, S.L. Simon, D.L. Preston, N. Hamada, M.S. Linet, C. Meyer, Occupational radiation exposure and risk of cataract incidence in a cohort of US radiologic technologists, *European journal of epidemiology*, 33 (2018) 1179-1191.

[222] E. Vano, N.J. Kleiman, A. Duran, M. Romano-Miller, M.M. Rehani, Radiation-associated lens opacities in catheterization personnel: results of a survey and direct assessments, *Journal of vascular and interventional radiology : JVIR*, 24 (2013) 197-204.

[223] S. Jacob, S. Boveda, O. Bar, A. Brezin, C. Maccia, D. Laurier, M.O. Bernier, Interventional cardiologists and risk of radiation-induced cataract: results of a French multicenter observational study, *International journal of cardiology*, 167 (2013) 1843-1847.

[224] G. Chodick, A.J. Sigurdson, R.A. Kleinerman, C.A. Sklar, W. Leisenring, A.C. Mertens, M. Stovall, S.A. Smith, R.E. Weathers, L.H. Veiga, L.L. Robison, P.D. Inskip, The Risk of Cataract among Survivors of Childhood and Adolescent Cancer: A Report from the Childhood Cancer Survivor Study, *Radiation research*, 185 (2016) 366-374.

[225] S. Bouffler, E. Ainsbury, P. Gilvin, J. Harrison, Radiation-induced cataracts: the Health Protection Agency's response to the ICRP statement on tissue reactions and recommendation on the dose limit for the eye lens, *Journal of radiological protection : official journal of the Society for Radiological Protection*, 32 (2012) 479-488.

[226] G. Chodick, N. Bekiroglu, M. Hauptmann, B.H. Alexander, D.M. Freedman, M.M. Doody, L.C. Cheung, S.L. Simon, R.M. Weinstock, A. Bouville, A.J. Sigurdson, Risk of cataract after exposure to low doses of ionizing radiation: a 20-year prospective cohort study among US radiologic technologists, *American journal of epidemiology*, 168 (2008) 620-631.

[227] T.V. Azizova, N. Hamada, E.S. Grigoryeva, E.V. Bragin, Risk of various types of cataracts in a cohort of Mayak workers following chronic occupational exposure to ionizing radiation., *European journal of epidemiology*, 33 (2018) in press.

[228] The 2007 Recommendations of the International Commission on Radiological Protection. ICRP publication 103, *Annals of the ICRP*, 37 (2007) 1-332.

- [229] L.T. Dauer, E.A. Ainsbury, J. Dynlacht, D. Hoel, B.E. Klein, D. Mayer, C.R. Prescott, R.H. Thornton, E. Vano, G.E. Woloschak, C.M. Flannery, L.E. Goldstein, N. Hamada, P.K. Tran, M.P. Grissom, E.A. Blakely, Status of NCRP Scientific Committee 1-23 Commentary on Guidance on Radiation Dose Limits for the Lens of the Eye, *Health physics*, 110 (2016) 182-184.
- [230] L.T. Dauer, E.A. Ainsbury, J. Dynlacht, D. Hoel, B.E.K. Klein, D. Mayer, C.R. Prescott, R.H. Thornton, E. Vano, G.E. Woloschak, C.M. Flannery, L.E. Goldstein, N. Hamada, P.K. Tran, M.P. Grissom, E.A. Blakely, Guidance on radiation dose limits for the lens of the eye: overview of the recommendations in NCRP Commentary No. 26, *International journal of radiation biology*, 93 (2017) 1015-1023.
- [231] L.T. Dauer, N. Hamada, E.A. Blakely, National Council on Radiation Protection and Measurements Commentary Number 26: Impact of Revised Guidance on Radiation Protection for the Lens of the Eye, *Journal of the American College of Radiology : JACR*, 14 (2017) 980-982.
- [232] D.G. Cogan, D.D. Donaldson, Experimental radiation cataracts. I. Cataracts in the rabbit following single x-ray exposure, *A.M.A. archives of ophthalmology*, 45 (1951) 508-522.
- [233] C. von Sonntag, The chemistry of free-radical-mediated DNA damage, *Basic life sciences*, 58 (1991) 287-317; discussion 317-221.
- [234] D. Sidjanin, S. Zigman, J. Reddan, DNA damage and repair in rabbit lens epithelial cells following UVA radiation, *Current eye research*, 12 (1993) 773-781.
- [235] B.L. Mahaney, K. Meek, S.P. Lees-Miller, Repair of ionizing radiation-induced DNA double-strand breaks by non-homologous end-joining, *Biochem J*, 417 (2009) 639-650.
- [236] W.B. Parsons, C.H. Watkins, G.L. Pease, D.S. Childs, Changes in sternal bone marrow following rontegan-ray therapy to the spleen in chronic granulocytic leukaemia, *Cancer*, 7 (1954) 179-189.
- [237] R.H. Mole, Whole body irradiation; radiobiology or medicine?, *The British journal of radiology*, 26 (1953) 234-241.
- [238] N. Hamada, M. Maeda, K. Otsuka, M. Tomita, Signaling pathways underpinning the manifestations of ionizing radiation-induced bystander effects, *Current molecular pharmacology*, 4 (2011) 79-95.
- [239] C. Mothersill, C. Seymour, Radiation-induced bystander effects: past history and future directions, *Radiation research*, 155 (2001) 759-767.
- [240] M. Kadhim, S. Salomaa, E. Wright, G. Hildebrandt, O.V. Belyakov, K.M. Prise, M.P. Little, Non-targeted effects of ionising radiation--implications for low dose risk, *Mutation research*, 752 (2013) 84-98.
- [241] V.M. Berthoud, E.C. Beyer, Oxidative stress, lens gap junctions, and cataracts, *Antioxidants & redox signaling*, 11 (2009) 339-353.

- [242] N. Hamada, Y. Fujimichi, Role of carcinogenesis related mechanisms in cataractogenesis and its implications for ionizing radiation cataractogenesis, *Cancer letters*, 368 (2015) 262-274.
- [243] M.A. Kadhim, S.R. Moore, E.H. Goodwin, Interrelationships amongst radiation-induced genomic instability, bystander effects, and the adaptive response, *Mutation research*, 568 (2004) 21-32.
- [244] J.H. Hayden, H. Rothstein, B.V. Worgul, G.R. Merriam, Jr., Hypophysectomy exerts a radioprotective effect on frog lens, *Experientia*, 36 (1980) 116-118.
- [245] Y. Fujimichi, N. Hamada, Ionizing irradiation not only inactivates clonogenic potential in primary normal human diploid lens epithelial cells but also stimulates cell proliferation in a subset of this population, *PloS one*, 9 (2014) e98154.
- [246] C. Hanna, J.E. O'Brien, Lens epithelial cell proliferation and migration in radiation cataracts, *Radiation research*, 19 (1963) 1-11.
- [247] E. Markiewicz, S. Barnard, J. Haines, M. Coster, O. van Geel, W. Wu, S. Richards, E. Ainsbury, K. Rothkamm, S. Bouffler, R.A. Quinlan, Nonlinear ionizing radiation-induced changes in eye lens cell proliferation, cyclin D1 expression and lens shape, 5 (2015) 150011.
- [248] N. Hamada, Ionizing radiation response of primary normal human lens epithelial cells, *PloS one*, 12 (2017) e0181530.
- [249] F. Supek, B. Lehner, Clustered Mutation Signatures Reveal that Error-Prone DNA Repair Targets Mutations to Active Genes, *Cell*, 170 (2017) 534-547.e523.
- [250] J. Frigola, R. Sabarinathan, L. Mularoni, F. Muinos, A. Gonzalez-Perez, N. Lopez-Bigas, Reduced mutation rate in exons due to differential mismatch repair, *Nature genetics*, 49 (2017) 1684-1692.
- [251] B. Schuster-Bockler, B. Lehner, Chromatin organization is a major influence on regional mutation rates in human cancer cells, *Nature*, 488 (2012) 504-507.
- [252] C.J. Bakkenist, M.B. Kastan, Chromatin perturbations during the DNA damage response in higher eukaryotes, *DNA Repair (Amst)*, 36 (2015) 8-12.
- [253] S.Y. Khan, S.F. Hackett, M.C. Lee, N. Pourmand, C.C. Talbot, Jr., S.A. Riazuddin, Transcriptome Profiling of Developing Murine Lens Through RNA Sequencing, *Investigative ophthalmology & visual science*, 56 (2015) 4919-4926.
- [254] H. Pan, A.E. Griep, Altered cell cycle regulation in the lens of HPV-16 E6 or E7 transgenic mice: implications for tumor suppressor gene function in development, *Genes & development*, 8 (1994) 1285-1299.
- [255] G.R. Cavaleiro, G.E. Matos-Rodrigues, Y. Zhao, A.L. Gomes, D. Anand, D. Predes, S. de Lima, J.G. Abreu, D. Zheng, S.A. Lachke, A. Cvekl, R.A.P. Martins, N-myc regulates

growth and fiber cell differentiation in lens development, *Developmental biology*, 429 (2017) 105-117.

[256] M. Petroni, F. Sardina, C. Heil, M. Sahun-Roncero, V. Colicchia, V. Veschi, S. Albini, D. Fruci, B. Ricci, A. Soriani, L. Di Marcotullio, I. Screpanti, A. Gulino, G. Giannini, The MRN complex is transcriptionally regulated by MYCN during neural cell proliferation to control replication stress, *Cell death and differentiation*, 23 (2016) 197-206.

[257] K. Bannik, U. Rossler, T. Faus-Kessler, M. Gomolka, S. Hornhardt, C. Dalke, O. Klymenko, M. Rosemann, K.R. Trott, M. Atkinson, U. Kulka, J. Graw, Are mouse lens epithelial cells more sensitive to gamma-irradiation than lymphocytes?, *Radiation and environmental biophysics*, 52 (2013) 279-286.

[258] C. Slingsby, G.J. Wistow, A.R. Clark, Evolution of crystallins for a role in the vertebrate eye lens, *Protein science : a publication of the Protein Society*, 22 (2013) 367-380.

[259] K.L. Moreau, J.A. King, Protein misfolding and aggregation in cataract disease and prospects for prevention, *Trends in molecular medicine*, 18 (2012) 273-282.

[260] N. Fujii, H. Uchida, T. Saito, The damaging effect of UV-C irradiation on lens alpha-crystallin, *Molecular vision*, 10 (2004) 814-820.

[261] I. Kim, T. Saito, N. Fujii, T. Kanamoto, T. Chatake, N. Fujii, Site specific oxidation of amino acid residues in rat lens gamma-crystallin induced by low-dose gamma-irradiation, *Biochemical and biophysical research communications*, 466 (2015) 622-628.

[262] LDLensRad, Towards a full mechanistic understanding of low dose radiation cataracts, CONCERT, http://www.concert-h2020.eu/-/media/Files/Concert/AIR2/Infrastructures_AIR2_Bulletin_Special_issue_Feb_2017.pdf?la=en&hash=DA73DD5DAF566F455BF0F2DE090135664333DACE, 2017, February.

[263] T. Nakazawa, S. Nagatsuka, Radiation-induced lipid peroxidation and membrane permeability in liposomes, *International journal of radiation biology and related studies in physics, chemistry, and medicine*, 38 (1980) 537-544.

[264] T. Micelli-Ferrari, G. Vendemiale, I. Grattagliano, F. Boscia, L. Arnese, E. Altomare, L. Cardia, Role of lipid peroxidation in the pathogenesis of myopic and senile cataract, *The British journal of ophthalmology*, 80 (1996) 840-843.

[265] C. Zerbinati, L. Iuliano, Cholesterol and related sterols autoxidation, *Free radical biology & medicine*, 111 (2017) 151-155.

[266] R.J. Truscott, X. Zhu, Presbyopia and cataract: a question of heat and time, *Progress in retinal and eye research*, 29 (2010) 487-499.

[267] S. Mrena, T. Kivela, P. Kurttio, A. Auvinen, Lens opacities among physicians occupationally exposed to ionizing radiation--a pilot study in Finland, *Scandinavian journal of work, environment & health*, 37 (2011) 237-243.

- [268] S. Lofgren, Solar ultraviolet radiation cataract, *Experimental eye research*, 156 (2017) 112-116.
- [269] K. Pirie, R. Peto, G.K. Reeves, J. Green, V. Beral, The 21st century hazards of smoking and benefits of stopping: a prospective study of one million women in the UK, *Lancet* (London, England), 381 (2013) 133-141.
- [270] I.D. Nicholl, A.W. Stitt, J.E. Moore, A.J. Ritchie, D.B. Archer, R. Bucala, Increased levels of advanced glycation endproducts in the lenses and blood vessels of cigarette smokers, *Molecular medicine* (Cambridge, Mass.), 4 (1998) 594-601.
- [271] B.S. Mamatha, B. Nidhi, C.A. Padmaprabhu, P. Pallavi, B. Vallikannan, Risk Factors for Nuclear and Cortical Cataracts: A Hospital Based Study, *Journal of ophthalmic & vision research*, 10 (2015) 243-249.
- [272] G.M. Richter, F. Choudhury, M. Torres, S.P. Azen, R. Varma, Risk factors for incident cortical, nuclear, posterior subcapsular, and mixed lens opacities: the Los Angeles Latino eye study, *Ophthalmology*, 119 (2012) 2040-2047.

Figure 1: The eye, the lens and the epithelium (A) The eye lens is located behind the cornea and in front of the retina. It is positioned in the eye by ligaments from the ciliary body and the hyaloid membrane by Wieger's ligament that then embed into the lens capsule. The hyaloid membrane separates the two humours of the eye, the aqueous and vitreous. **(B)** Schematic of the epithelium that covers the anterior hemisphere of the eye lens. The epithelium has different zones, the central, germinative and transitional zones. The density of the cells (blue dots) changes across the epithelium. Epithelial cell proliferation is greater in the germinative zone (dividing cells represented by red dots). Cells in this region embark on their programme of differentiation by moving into the transitional zone and then into the meridional rows where they become organized ready to form lens fiber cells. The meridional rows are highly organized, but with age this organization declines. **(C)** An example of a mouse lens stained with DAPI to identify cell nuclei in the lens epithelium. The lens is orientated in the same way as the schematic. Notice the change in cell density between the different zones and the organization in the meridional rows.

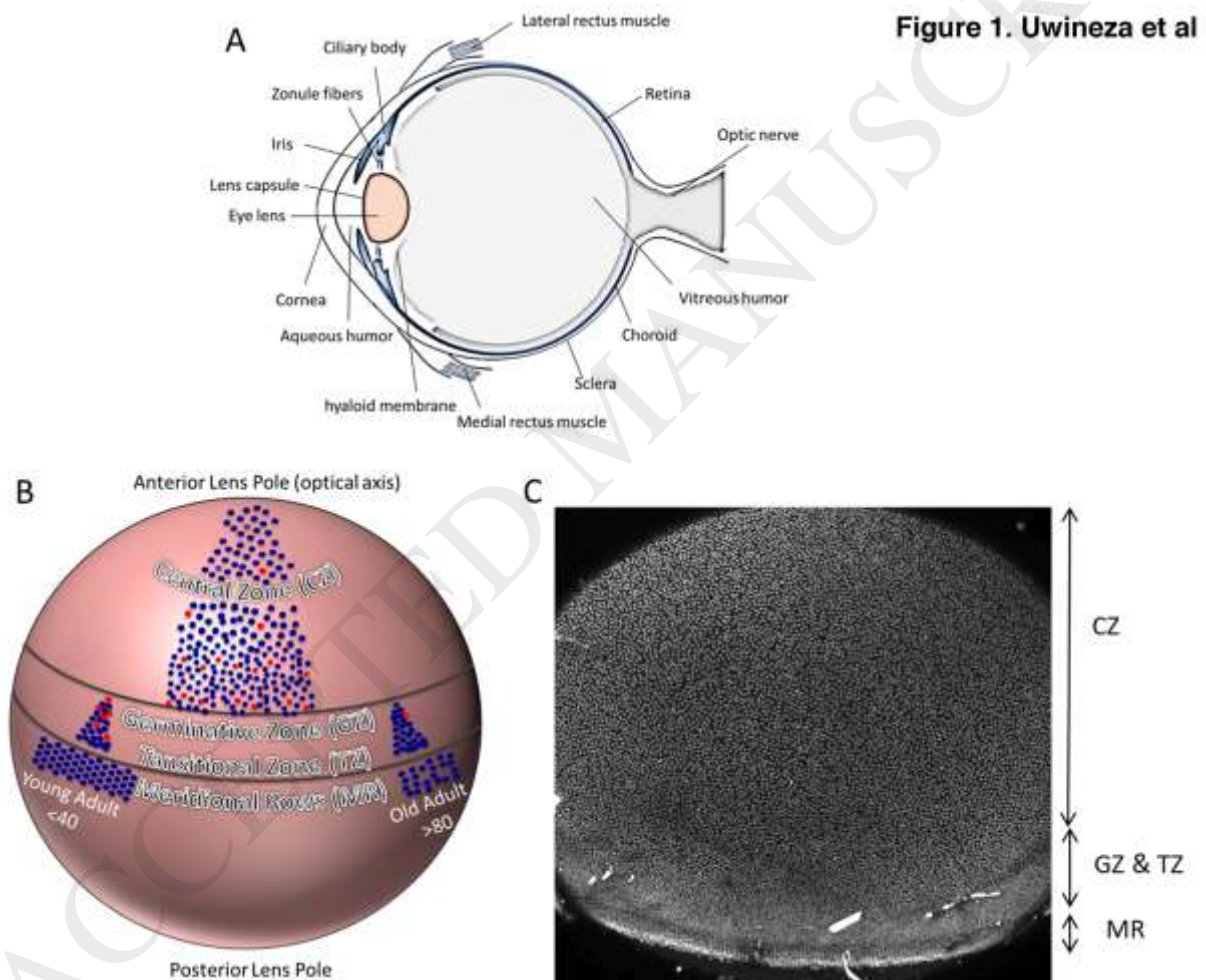


Figure 2. Lifelong cataractogenic load accumulation and the latency of cataract (A) Timeline for lens aging and the appearance of cataract under the influence of the accumulated cataractogenic load. Aging of the lens is dependent upon genetic, environmental and stochastic factors. A universal aging effect upon the lens is the development of presbyopia at the end of the fourth decade of life. The appearance of cataract depends upon the accumulated cataractogenic load, which is specific to each individual. Age related cataract (ARC) is phenotypically variable, although the most frequent ARC is a nuclear cataract. The additional burden of IR exposure can accelerate

cataractogenesis, but there can be a long latency period between the exposure and the resulting cataract. Where cataract incidence involving an IR event has been studied, these have almost entirely involved younger people (<30) and whilst PSC is a common cataract phenotype as a result of acute IR exposure, cortical and nuclear cataracts are also seen after low dose IR exposure. **(B)** The accumulated, cataractogenic load determines when cataract will appear in the lens of an individual. This cataractogenic load accumulates differently for each individual dependent upon the genetic, environmental and stochastic factors. Simplistically this is represented as a threshold, but for a population this threshold is reached at different ages as reflected in wide age range of cataract onset. **(C)** IR adds to the cataractogenic load and therefore for the individual represented in (B), the threshold for cataract formation is reached earlier because the rate has increased.

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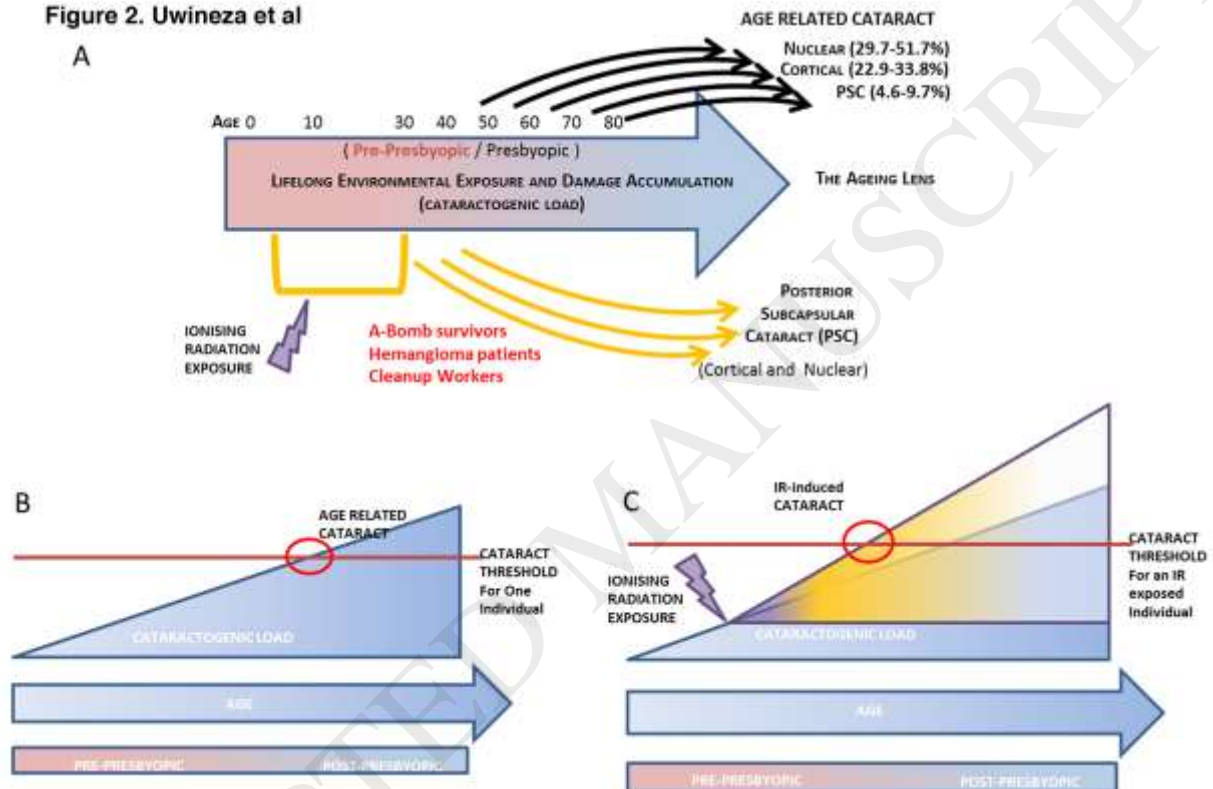


Figure 3: IR induced damage to the genome, proteome and lipidome of lens cells. The two cell types in the lens, Lens Epithelial Cells (LECs) and Lens Fiber Cells (LFCs), are both sensitive to ionizing radiation (IR). IR can cause the production of Reactive Oxygen Species (ROS) from water molecules. ROS causes double stranded breaks (DSBs) in the DNA within LECs and LFCs. ROS can also trigger protein modifications that lead to protein aggregation and the oxidation of lipids in the membranes of both LECs and LFCs.

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